```
=> b reg
FILE 'REGISTRY' ENTERED AT 17:10:28 ON 08 JUL 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 American Chemical Society (ACS)

Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES:    7 JUL 2004  HIGHEST RN 705925-25-3
DICTIONARY FILE UPDATES:   7 JUL 2004  HIGHEST RN 705925-25-3

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 6, 2004

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available. For more
information enter HELP PROP at an arrow prompt in the file or refer
to the file summary sheet on the web at:
http://www.cas.org/ONLINE/DBSS/registryss.html

=> => d que l1
L1             4 SEA FILE=REGISTRY ABB=ON  PLU=ON  "TISSUE FACTOR PATHWAY
                 INHIBITOR"/CN OR "TISSUE FACTOR PATHWAY INHIBITOR (HUMAN)"/CN
                 OR "TISSUE FACTOR PATHWAY INHIBITOR-2 (HUMAN)"/CN


=> d ide l1 1-4

L1   ANSWER 1 OF 4  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   512861-40-4  REGISTRY
CN   Proteinase inhibitor, TFPI (human) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   1: PN: WO03032904 SEQID: 1 claimed protein
CN   Tissue factor pathway inhibitor (human)
FS   PROTEIN SEQUENCE
MF   Unspecified
CI   MAN
SR   CA
LC   STN Files:   CA, CAPLUS, TOXCENTER, USPATFULL
DT.CA  CAplus document type:  Patent
RL.P   Roles from patents:  BIOL (Biological study); PRP (Properties)

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               1 REFERENCES IN FILE CA (1907 TO DATE)
               1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1   ANSWER 2 OF 4  REGISTRY  COPYRIGHT 2004 ACS on STN
RN   481228-88-0  REGISTRY
CN   Tissue factor pathway inhibitor-2 (human) (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   GenBank AAA20094
CN   GenBank AAA20094 (Translated from: GenBank L27624)
FS   PROTEIN SEQUENCE
```

MF Unspecified
CI MAN
SR GenBank
LC STN Files: CA, CAPLUS, USPATFULL
DT.CA CAplus document type: Patent
RL.P Roles from patents: BIOL (Biological study); PRP (Properties)

RELATED SEQUENCES AVAILABLE WITH SEQLINK

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
1 REFERENCES IN FILE CA (1907 TO DATE)
1 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 3 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 194554-71-7 REGISTRY
CN Proteinase inhibitor, TFPI (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Blood-coagulation factors, EPI (extrinsic pathway inhibitor)
CN Blood-coagulation factors, LACI
CN Blood-coagulation factors, lipoprotein-assocd. coagulation inhibitors
CN Blood-coagulation factors, TFI
CN EPI blood-coagulation factors
CN Extrinsic pathway inhibitor blood-coagulation factors
CN LACI blood-coagulation factors
CN Lipoprotein-assocd. coagulation inhibitor
CN Lipoprotein-assocd. coagulation inhibitors blood-coagulation factors
CN Tissue factor inhibitor
CN **Tissue factor pathway inhibitor**
MF Unspecified
CI MAN
SR CA
LC STN Files: BIOSIS, CA, CAPLUS, IPA, TOXCENTER, USPAT2, USPATFULL
DT.CA CAplus document type: Conference; Dissertation; Journal; Patent
RL.P Roles from patents: ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); USES (Uses)
RLD.P Roles for non-specific derivatives from patents: BIOL (Biological study); PREP (Preparation); PROC (Process); PRP (Properties); RACT (Reactant or reagent); USES (Uses)
RL.NP Roles from non-patents: ANST (Analytical study); BIOL (Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence); PREP (Preparation); PROC (Process); PRP (Properties); USES (Uses)
RLD.NP Roles for non-specific derivatives from non-patents: BIOL (Biological study); OCCU (Occurrence); PROC (Process); PRP (Properties); USES (Uses)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
584 REFERENCES IN FILE CA (1907 TO DATE)
16 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
585 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L1 ANSWER 4 OF 4 REGISTRY COPYRIGHT 2004 ACS on STN
RN 170276-44-5 REGISTRY
CN Blood-coagulation factor LACI [alanyl] (human clone λP9) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN **Tissue factor pathway inhibitor (human)**
FS PROTEIN SEQUENCE
MF Unspecified
CI MAN

```
SR   CA
LC   STN Files:   CA, CAPLUS, TOXCENTER
DT.CA  CAplus document type:  Journal; Patent
RL.P   Roles from patents:  ANST (Analytical study); BIOL (Biological study);
       USES (Uses)
RL.NP  Roles from non-patents:  PRP (Properties)

**RELATED SEQUENCES AVAILABLE WITH SEQLINK**

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
*** USE 'SQD' OR 'SQIDE' FORMATS TO DISPLAY SEQUENCE ***
               2 REFERENCES IN FILE CA (1907 TO DATE)
               2 REFERENCES IN FILE CAPLUS (1907 TO DATE)


=>
```

```
=> b hcaplus
FILE 'HCAPLUS' ENTERED AT 17:12:25 ON 08 JUL 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)
```



```
FILE COVERS 1907 - 8 Jul 2004  VOL 141 ISS 2
FILE LAST UPDATED: 7 Jul 2004  (20040707/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

'OBI' IS DEFAULT SEARCH FIELD FOR 'HCAPLUS' FILE

=> => d que l59
L50 (         4) SEA FILE=REGISTRY ABB=ON  PLU=ON  "TISSUE FACTOR PATHWAY
                   INHIBITOR"/CN OR "TISSUE FACTOR PATHWAY INHIBITOR (HUMAN)"/CN
                   OR "TISSUE FACTOR PATHWAY INHIBITOR-2 (HUMAN)"/CN
L51 (       588) SEA FILE=HCAPLUS ABB=ON  PLU=ON  L50
L52 (     11206) SEA FILE=HCAPLUS ABB=ON  PLU=ON  "PROTEIN FOLDING"/CT
L53 (    291669) SEA FILE=HCAPLUS ABB=ON  PLU=ON  SOLUBILIZATION/CT OR ?SOLUBIL?
                   /BI
L54 (      5904) SEA FILE=HCAPLUS ABB=ON  PLU=ON  PURIFICATION/CT
L55 (       327) SEA FILE=HCAPLUS ABB=ON  PLU=ON  (BLOOD COAGULATION FACTOR?/OBI
                   ) (3A) (EXTRINSIC/OBI OR LACI/OBI OR EPI/OBI OR LIPOPROTEIN?/OBI)

L56 (       253) SEA FILE=HCAPLUS ABB=ON  PLU=ON  (COAGULATION INHIBITOR?/OBI) (3
                   A) (LIPOPROTEIN/OBI OR LACI/OBI OR EXTRINISIC/OBI)
L57 (       713) SEA FILE=HCAPLUS ABB=ON  PLU=ON  TFPI/OBI OR L51
L58 (       984) SEA FILE=HCAPLUS ABB=ON  PLU=ON  L55 OR L56 OR L57
L59           8  SEA FILE=HCAPLUS ABB=ON  PLU=ON  (L53 OR L52 OR L54) AND L58


=> b ipa
FILE 'IPA' ENTERED AT 17:13:11 ON 08 JUL 2004
COPYRIGHT (C) 2004 American Society of Hospital Pharmacists (ASHP)

FILE COVERS 1970 TO 1 JUL 2004 (20040701/ED)

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> => d que l63
L60 (      3295) SEA FILE=IPA ABB=ON  PLU=ON  SOLUBILITY/CT
L61 (         3) SEA FILE=REGISTRY ABB=ON  PLU=ON  "TISSUE FACTOR PATHWAY
                   INHIBITOR"/CN OR "TISSUE FACTOR PATHWAY INHIBITOR (HUMAN)"/CN
L62 (         5) SEA FILE=IPA ABB=ON  PLU=ON  L61
L63           1  SEA FILE=IPA ABB=ON  PLU=ON  L60 AND L62
```

```
=> b biosis
FILE 'BIOSIS' ENTERED AT 17:13:59 ON 08 JUL 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 7 July 2004 (20040707/ED)

FILE RELOADED: 19 October 2003.

=> => d que l49
L1             4 SEA FILE=REGISTRY ABB=ON  PLU=ON  "TISSUE FACTOR PATHWAY
                   INHIBITOR"/CN OR "TISSUE FACTOR PATHWAY INHIBITOR (HUMAN)"/CN
                   OR "TISSUE FACTOR PATHWAY INHIBITOR-2 (HUMAN)"/CN
L19          539 SEA FILE=BIOSIS ABB=ON  PLU=ON  L1
L20           46 SEA FILE=BIOSIS ABB=ON  PLU=ON  TISSUE FACTOR INHIBITOR?
L21          571 SEA FILE=BIOSIS ABB=ON  PLU=ON  L19 OR L20
L22          125 SEA FILE=BIOSIS ABB=ON  PLU=ON  (BLOOD(W)COAGULATION)(3A)(EPI
                   OR EXTRINSIC OR LACI OR TFI)
L23          680 SEA FILE=BIOSIS ABB=ON  PLU=ON  L21 OR L22
L24            1 SEA FILE=BIOSIS ABB=ON  PLU=ON  EXTRINISIC(W)PATHWAY(W)INHIBITO
                   R?
L25          681 SEA FILE=BIOSIS ABB=ON  PLU=ON  L24 OR L23
L32       483873 SEA FILE=BIOSIS ABB=ON  PLU=ON  ?PURIF? OR ?SOLUBIL? OR
                   ?FORMULA?
L47         1191 SEA FILE=BIOSIS ABB=ON  PLU=ON  TFPI OR L25
L48           80 SEA FILE=BIOSIS ABB=ON  PLU=ON  L32 AND L47
L49           28 SEA FILE=BIOSIS ABB=ON  PLU=ON  METHOD? AND L48


=> b wpix
FILE 'WPIX' ENTERED AT 17:14:58 ON 08 JUL 2004
COPYRIGHT (C) 2004 THOMSON DERWENT

FILE LAST UPDATED:          2 JUL 2004      <20040702/UP>
MOST RECENT DERWENT UPDATE:     200442        <200442/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
 http://www.stn-international.de/training_center/patents/stn_guide.pdf <<<

>>> FOR DETAILS OF THE PATENTS COVERED IN CURRENT UPDATES, SEE
    http://thomsonderwent.com/coverage/latestupdates/           <<<

>>> FOR INFORMATION ON ALL DERWENT WORLD PATENTS INDEX USER
    GUIDES, PLEASE VISIT:
    http://thomsonderwent.com/support/userguides/               <<<

>>> NEW! FAST-ALERTING ACCESS TO NEWLY-PUBLISHED PATENT
    DOCUMENTATION NOW AVAILABLE IN DERWENT WORLD PATENTS INDEX
    FIRST VIEW - FILE WPIFV. FREE CONNECT HOUR UNTIL 1 MAY 2004.
    FOR FURTHER DETAILS: http://www.thomsonderwent.com/dwpifv  <<<

>>> NEW! IMPROVE YOUR LITIGATION CHECKING AND INFRINGEMENT
    MONITORING WITH LITALERT. FIRST ACCESS TO RECORDS OF IP
    LAWSUITS FILED IN THE 94 US DISTRICT COURTS SINCE 1973.
```

```
    FOR FURTHER DETAILS:
    http://www.thomsonscientific.com/litalert          <<<

>>> THE DISPLAY LAYOUT HAS BEEN CHANGED TO ACCOMODATE THE
    NEW FORMAT GERMAN PATENT APPLICATION AND PUBLICATION
    NUMBERS. SEE ALSO:
    http://www.stn-international.de/archive/stnews/news0104.pdf <<<

=> => d que l72
L64 (        87) SEA FILE=WPIX ABB=ON  PLU=ON  TISSUE(W)FACTOR(W)PATHWAY(W)INHIB
                 ITOR/BIX
L65 (         7) SEA FILE=WPIX ABB=ON  PLU=ON  (BLOOD(W)COAGULATION(3A)(EPI OR
                 LACI OR TFI OR EXTRINSIC OR LIPOPROTEIN?))/BIX
L66 (        94) SEA FILE=WPIX ABB=ON  PLU=ON  L64 OR L65
L67 (    108459) SEA FILE=WPIX ABB=ON  PLU=ON  PURIFICATION/BIX
L68 (    876551) SEA FILE=WPIX ABB=ON  PLU=ON  (?SOLUB? OR ?FORMULA?)/BIX
L69 (    176967) SEA FILE=WPIX ABB=ON  PLU=ON  PURIF?/BIX
L70 (    176967) SEA FILE=WPIX ABB=ON  PLU=ON  L67 OR L69
L71 (   1015415) SEA FILE=WPIX ABB=ON  PLU=ON  L70 OR L68
L72          35 SEA FILE=WPIX ABB=ON  PLU=ON  L71 AND L66


=> => => b embase
FILE 'EMBASE' ENTERED AT 17:17:49 ON 08 JUL 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

 FILE COVERS 1974 TO 1 Jul 2004 (20040701/ED)

 EMBASE has been reloaded. Enter HELP RLOAD for details.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> => d que l80
L73 (       689) SEA FILE=EMBASE ABB=ON  PLU=ON  ANTITHROMBOPLASTIN/CT OR
                 "EXTRINSIC COAGULATION PATHWAY INHIBITOR"/CT OR "LIPOPROTEIN
                 ASSOCIATED COAGULATION INHIBITOR"/CT OR TFPI
L74 (      6435) SEA FILE=EMBASE ABB=ON  PLU=ON  PURIFICATION/CT
L75 (     24210) SEA FILE=EMBASE ABB=ON  PLU=ON  "PROTEIN PURIFICATION"/CT
L76 (     30595) SEA FILE=EMBASE ABB=ON  PLU=ON  L74 OR L75
L77 (        13) SEA FILE=EMBASE ABB=ON  PLU=ON  L73 AND L76
L78 (      3653) SEA FILE=EMBASE ABB=ON  PLU=ON  SOLUBILIZATION/CT
L79 (     55767) SEA FILE=EMBASE ABB=ON  PLU=ON  ?SOLUBIL? OR L78
L80           2 SEA FILE=EMBASE ABB=ON  PLU=ON  L77 AND L79


=> dup rem l49 l63 l80 l59 l72
FILE 'BIOSIS' ENTERED AT 17:18:38 ON 08 JUL 2004
COPYRIGHT (C) 2004 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'IPA' ENTERED AT 17:18:38 ON 08 JUL 2004
COPYRIGHT (C) 2004 American Society of Hospital Pharmacists (ASHP)

FILE 'EMBASE' ENTERED AT 17:18:38 ON 08 JUL 2004
COPYRIGHT (C) 2004 Elsevier Inc. All rights reserved.

FILE 'HCAPLUS' ENTERED AT 17:18:38 ON 08 JUL 2004
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)
```

FILE 'WPIX' ENTERED AT 17:18:38 ON 08 JUL 2004
COPYRIGHT (C) 2004 THOMSON DERWENT
PROCESSING COMPLETED FOR L49
PROCESSING COMPLETED FOR L63
PROCESSING COMPLETED FOR L80
PROCESSING COMPLETED FOR L59
PROCESSING COMPLETED FOR L72
L81 67 DUP REM L49 L63 L80 L59 L72 (7 DUPLICATES REMOVED)

=> => => d all l81 2 5 12 14 18 20 21 22 23 26 36 40 41 43 44 45 46 47 50 51 54 56 57 60 62 63 65 67
YOU HAVE REQUESTED DATA FROM FILE 'BIOSIS, HCAPLUS, IPA, WPIX' - CONTINUE? (Y)/N:y

L81 ANSWER 2 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2004:110294 BIOSIS
DN PREV200400112428
TI **Purification**, molecular cloning, and expression of a novel growth-promoting factor for retinal pigment epithelial cells, REF-1/**TFPI**-2.
AU Tanaka, Yasuhiko [Reprint Author]; Utsumi, Jun; Matsui, Mizuo; Sudo, Tetsuo; Nakamura, Noriko; Mutoh, Masato; Kajita, Akemi; Sone, Saburo; Kigasawa, Kazuteru; Shibuya, Masahiko; Reddy, Venkat N.; Zhang, Qiang; Iwata, Takeshi
CS National Institute of Sensory Organs, National Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro-ku, Tokyo, 152-8902, Japan
ytanaka@ntmc.hosp.go.jp
SO IOVS, (January 2004) Vol. 45, No. 1, pp. 245-252. print.
DT Article
LA English
ED Entered STN: 25 Feb 2004
Last Updated on STN: 25 Feb 2004
AB PURPOSE: Retinal pigment epithelial (RPE) cells are known to play important roles in maintaining the homeostasis of the retina and in controlling choroidal neovascularization. The purpose of this study was to identify a factor or factors that would stimulate RPE cells to proliferate. **METHODS**: To isolate such a factor, 100 L of human-fibroblast-conditioned medium underwent ion-exchange, hydrophobic, and reverse-phase chromatographies followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The growth-promoting activity of the factor was examined in a human K-1034 RPE cell line and human primary RPE cells. RESULTS: The different chromatographic processes isolated a 31-kDa factor that had RPE cell growth-promoting properties. This factor, which we have named RPE cell factor (REF)-1, promotes growth of RPE cells but not of human umbilical vein endothelial cells (HUVECs). The amino-terminal sequence and molecular cloned cDNA of REF-1 were identical with those of tissue-factor pathway inhibitor (**TFPI**)-2, a family of **TFPIs**, and placental protein (PP)-5, a serine protease inhibitor. The cDNA expression of REF-1/**TFPI**-2 with pcDL-pSRalpha vector in Chinese hamster ovary (CHO) cells confirmed the growth-promoting activity for RPE cells. The major component of the recombinant REF-1/**TFPI**-2 expressed in CHO cells had a molecular mass of 31 kDa and exerted growth-promoting activity in RPE cells but not in human endothelial cells and fibroblasts in vitro. REF-1/**TFPI**-2 also had protease inhibitory activity. The other family factor, **TFPI**-1, did not promote RPE cell growth. CONCLUSIONS: REF-1/**TFPI**-2 is a novel growth-promoting factor for RPE cells but not for endothelial cells and fibroblasts. Its properties make it potentially

beneficial for intraocular therapy for the repair and maintenance of RPE cells.

CC Cytology - Animal 02506
Cytology - Human 02508
Biochemistry studies - Nucleic acids, purines and pyrimidines 10062
Cardiovascular system - Physiology and biochemistry 14504
Sense organs - Physiology and biochemistry 20004

IT Major Concepts
Methods and Techniques; Sense Organs (Sensory Reception)

IT Parts, Structures, & Systems of Organisms
endothelial cells: circulatory system; fibroblast; retinal pigment epithelial cells: sensory system, growth, proliferation

IT Chemicals & Biochemicals
cDNA [complementary DNA]; placental protein-5 [PP-5]: serine protease inhibitor; retinal pigment epithelial cell factor-1 [REF-1]: amino-terminal sequence, growth promoting factor, expression; tissue factor pathway inhibitor-2 [**TFPI**-2]: growth promoting factor, expression

IT Methods & Equipment
SDS-polyacrylamide gel electrophoresis: electrophoretic techniques, laboratory techniques; hydrophobic chromatography: chromatographic techniques, laboratory techniques; ion-exchange chromatography: chromatographic techniques, laboratory techniques; reverse-phase chromatography: chromatographic techniques, laboratory techniques

IT Miscellaneous Descriptors
cell growth regulation

ORGN Classifier
Cricetidae 86310
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
CHO cell line (cell line): Chinese hamster ovary cells
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HUVEC cell line (cell line): human umbilical vein endothelial cells
K-1034 cell line (cell line): human retinal pigment epithelial cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 160477-63-4 (tissue factor pathway inhibitor-2)
160477-63-4 (**TFPI**-2)

L81 ANSWER 5 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2003-421522 [39] WPIX
CR 2003-513976 [48]; 2003-854085 [79]
DNN N2003-336632 DNC C2003-111114
TI Identifying mammalian cell capable of producing a proteinaceous molecule, by analyzing post-translational modification on a protein produced by mammalian cell, and determining whether protein comprises the modification.
DC B04 D16 S03
IN BOUT, A; BRUS, R H P; KAPTEYN, J C; OPSTELTEN, D J E; PASSIER, P C J J
PA (CRUC-N) CRUCELL HOLLAND BV
CYC 101
PI WO 2003038100 A1 20030508 (200339)* EN 174 C12N015-65

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW

ADT WO 2003038100 A1 WO 2002-NL686 20021029

PRAI WO 2002-NL257 20020419; WO 2001-NL792 20011029

IC ICM C12N015-65
ICS C07K014-505; C12N015-86; G01N033-68; G01N033-74

AB WO2003038100 A UPAB: 20031208
NOVELTY - Identifying (M1) a mammalian cell capable of producing a proteinaceous molecule having a predetermined post-translational modification, comprising analyzing the post- translational modification on a protein produced by the mammalian cell, and determining whether the protein comprises the predetermined post-translational modification, is new.

DETAILED DESCRIPTION - Identifying (M1) a mammalian cell capable of producing a proteinaceous molecule having a predetermined post-translational modification, comprising:

(a) analyzing the post- translational modification on a protein produced by the mammalian cell; and

(b) determining whether the protein comprises the predetermined post-translational modification.

The mammalian cell is selected which is capable of producing a proteinaceous molecule, by analyzing the presence or absence of a tissue specific marker or a combination of tissue specific markers in the mammalian cell or on the cell surface of the mammalian cell, which marker or combination of the markers is indicative for the predetermined post-translational modification to be present on the proteinaceous molecule, and selecting the mammalian cell on the basis of the presence or absence of the tissue specific markers. The mammalian cell is obtained from a heterogeneous cell population, the mammalian cell being capable of producing a proteinaceous molecule, by sorting cells on the basis of the post-translational modifications on proteins produced by the cells in the heterogeneous cell population, and selecting the cells capable of producing proteins comprising the predetermined post-translational modification. INDEPENDENT CLAIMS are also included for the following:

(1) identifying, and/or selecting, and/or obtaining a mammalian cell capable of producing a proteinaceous molecule comprising a predetermined post-translational modification of M1, where the predetermined post-translational modifications are present on a proteinaceous molecule that is recombinantly expressed in the mammalian cell;

(2) a pharmaceutical composition (I) comprising recombinantly produced erythropoietin having a predetermined post-translational modification, where the recombinantly produced erythropoietin is obtained using M1, and has a lower erythropoietic effect as compared to erythropoietin not having the predetermined post-translational modification, and a carrier;

(3) recombinantly produced erythropoietin (II) comprising at least one post-translational modification selected from sialyl Lewis x structure, Lewis x structure, alpha ,3- linked fucose attached to N-acetyl-glucosamine, LacidNAc structure, terminal N-acetyl-glucosamine group and a terminal galactose group;

(4) use of a mammalian cell obtainable by M1 for the production of a proteinaceous molecule comprising a predetermined post-translational modification;

(5) a pharmaceutical preparation comprising erythropoietin-like molecules selected from erythropoietin, one or more muteins of

erythropoietin, one or more derivatives of erythropoietin, and a composition of one or more fractions of erythropoietin or erythropoietin-like molecules sialylated to a varying degree;

(6) preventative and/or therapeutic treatment of a disorder chosen from ischemia, a reperfusion injury, a hypoxia-induced disorder, an inflammatory disease, neurodegenerative disorder, and acute damage to the central-or peripheral nervous system, comprising administering to a human or animal subject, a protein content basis a lower erythropoietic activity in vivo than epoetin alfa, and/or the presence of erythropoietin-like molecules that once administered parenterally to a human or an animal subject are cleared from the bloodstream at a faster rate than epoetin alfa;

(7) producing in a mammalian cell proteinaceous molecules in need of a glycosylation structure chosen from (sialyl) Lewis X and/or LacdiNac containing N-linked glycan structures, characterized in that the cell expresses nucleic acid encoding E1A from an adenovirus, with the proviso that when the proteinaceous molecule is erythropoietin the mammalian cell is not a PER.C6 (RTM) cell, when the proteinaceous molecule is protein C the mammalian cell is not a HEK293 cell or a Syrian hamster AV12-664 cell, when the proteinaceous molecule is glycodelin or **tissue factor pathway inhibitor** the mammalian cell is not a HEK293 cell, and when the proteinaceous molecule is matrix metalloprotease 1 the mammalian cell is not a HT1080 cell;

(8) producing a fraction enriched in a proteinaceous molecule having N-linked glycans comprising (sialyl)Lewis X and/or LacdiNac structures, by recombinantly expressing the proteinaceous molecule in a cell that expresses nucleic acid encoding E1A from an adenovirus, and fractionating the proteinaceous molecules so produced, thus obtaining a fraction which is enriched in molecules having the N-linked glycans comprising (sialyl)Lewis X and/or LacidNac structures;

(9) fractionating a mixture comprising proteinaceous molecules that comprise Lewis X structures, the method employing binding of the molecules to an AAL lectin;

(10) a composition comprising erythropoietin-like molecules chosen from erythropoietin, one or more muteins of erythropoietin, and one or more derivatives of erythropoietin, characterized in that the average number of lewis-X structures on N-linked glycans per erythropoietin-like molecule is at least 2.2; and

(11) a composition of erythropoietin-like molecules is characterized in that it is recombinantly producible in a mammalian cell comprising nucleic acid encoding E1A from an adenovirus.

ACTIVITY - Vasotropic; Antiinflammatory.

No biological data is given.

MECHANISM OF ACTION - None given.

USE - M1 is useful for identifying, selecting, obtaining a mammalian cell capable of producing a proteinaceous molecule comprising a predetermined post-translational modification, where the mammalian cell is of neural origin, human cell, or immortalized. The mammalian cell has been provided with a nucleic acid encoding the E1 region, or its part, from human adenovirus in such a way that the mammalian cell harbors the nucleic acid in an expressible form. The proteinaceous molecule is erythropoietin. M1 is also useful for producing a proteinaceous molecule and expressing the proteinaceous molecule in the mammalian cell. The mammalian cell is PER.C6 (RTM). The extra step of **purifying** the proteinaceous molecule from the mammalian cell culture. The **purification** comprises a step employs the predetermined post- translational modification. The **purification** comprises a step in which an antibody is employed that is specific for an epitope present modification, and comprises a lectin-binding step. (I) is useful for the preparation of a medicament for the treatment of a disorder selected from ischemia,

reperfusion injury, hypoxia-induced disorder, inflammatory disease, neurodegenerative disorder, and acute damage to the central-or peripheral nervous system. (All claimed.)
Dwg.0/26

FS CPI EPI
FA AB; DCN
MC CPI: B04-F02; B04-F0200E; B04-H0700E; B04-N02; B11-C07B; B11-C08E1; B12-K04E; B14-C03; B14-F02D; B14-F05; B14-J01; D05-C12; D05-H08; D05-H09; D05-H14B2; D05-H17A2
EPI: S03-E14H

L81 ANSWER 12 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2002-643334 [69] WPIX
CR 2002-643328 [69]; 2002-643332 [69]; 2002-643333 [69]; 2002-691526 [74]
DNC C2004-014190
TI Composition useful for e.g. treating vascular conditions (hyperlipidemia), diabetes, obesity or lowering a concentration of a sterol in plasma of a mammal, comprises sterol absorption inhibitor and blood modifier.
DC B03 B05
IN KOSOGLOU, T; RESS, R J; STRONY, J; VELTRI, E P; STRONY, J T
PA (SCHE) SCHERING CORP
CYC 98
PI WO 2002058734 A2 20020801 (200269)* EN 103 A61K045-06
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CZ DE DK DM DZ EC EE ES FI GB GD GE HR HU ID IL IN IS JP KG KR KZ LC LK LR LT LU LV MA MD MG MK MN MX MZ NO NZ PH PL PT RO RU SE SG SI SK SL TJ TM TN TR TT TZ UA UZ VN YU ZA ZM
US 2002147184 A1 20021010 (200269) A61K031-397
EP 1353694 A2 20031022 (200370) EN A61K045-06
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
NO 2003003357 A 20030925 (200373) A61K045-06
SK 2003000950 A3 20031201 (200404) A61K045-06
BR 2002006639 A 20040225 (200416) A61K045-06
HU 2003003917 A2 20040301 (200422) A61K045-06
AU 2002237927 A1 20020806 (200427) A61K045-06
CZ 2003002039 A3 20040114 (200429) A61K045-06
JP 2004517920 W 20040617 (200440) 192 A61K045-06
ADT WO 2002058734 A2 WO 2002-US2013 20020125; US 2002147184 A1 Provisional US 2001-264275P 20010126, Provisional US 2001-264396P 20010126, Provisional US 2001-264600P 20010126, Provisional US 2001-324123P 20010921, US 2002-56680 20020125; EP 1353694 A2 EP 2002-704233 20020125, WO 2002-US2013 20020125; NO 2003003357 A WO 2002-US2013 20020125, NO 2003-3357 20030725; SK 2003000950 A3 WO 2002-US2013 20020125, SK 2003-950 20020125; BR 2002006639 A BR 2002-6639 20020125, WO 2002-US2013 20020125; HU 2003003917 A2 WO 2002-US2013 20020125, HU 2003-3917 20020125; AU 2002237927 A1 AU 2002-237927 20020125; CZ 2003002039 A3 WO 2002-US2013 20020125, CZ 2003-2039 20020125; JP 2004517920 W JP 2002-559068 20020125, WO 2002-US2013 20020125
FDT EP 1353694 A2 Based on WO 2002058734; SK 2003000950 A3 Based on WO 2002058734; BR 2002006639 A Based on WO 2002058734; HU 2003003917 A2 Based on WO 2002058734; AU 2002237927 A1 Based on WO 2002058734; CZ 2003002039 A3 Based on WO 2002058734; JP 2004517920 W Based on WO 2002058734
PRAI US 2001-324123P 20010921; US 2001-264275P 20010126;
US 2001-264396P 20010126; US 2001-264600P 20010126;
US 2002-56680 20020125
IC ICM A61K031-397; A61K045-06
ICS A61K031-395; A61K031-69; A61K031-7052; A61P003-04; A61P003-06;

A61P003-10; A61P009-00; A61P009-10; A61P043-00

AB WO 200258734 A UPAB: 20040624
NOVELTY - A composition comprises at least one sterol absorption inhibitor and at least one blood modifier.
ACTIVITY - Antilipemic; Antidiabetic; Anorectic; Antiarteriosclerotic; Hypotensive; Antiinflammatory; Cerebroprotective; Antianginal; Cardiant; Anticoagulant.
MECHANISM OF ACTION - Sterol absorption inhibitor; Platelet function inhibitor.
USE - For the treatment or prevention of vascular condition (hyperlipidemia), diabetes, obesity or lowering a concentration of a sterol in plasma of a mammal (all claimed). Also for treating atherosclerosis, hypercholesterolemia, hypertriglyceridaemia, sitosterolemia, vascular inflammation, hypertension, angina, cardiac arrhythmias or stroke.
ADVANTAGE - By using combination therapy, the side effects of individual compounds can be reduced compared to monotherapy, which improves patient compliance and provides a broader range of complimentary effects or modes of action.
Dwg.0/0

FS CPI

FA AB; GI; DCN

MC CPI: B03-L; B04-C01A; B04-C01B; B04-C01D; B04-C02E1; B04-C03; B04-G21; B06-H; B07-D01; B07-H; B10-A17; B10-B02A; B10-C03; B10-C04A; B10-C04C; B10-H02A; B14-D02A2; B14-D05D; B14-E12; B14-F02; B14-F06; B14-F07; B14-N16; B14-S04; B14-S08

L81 ANSWER 14 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2002-610270 [66] WPIX

DNC C2002-172733

TI Pharmaceutical composition for treating systemic inflammatory response syndrome, sepsis, septic shock and/or thrombus formation in microvasculature in mammals, comprises a partial inhibitor of factor VIII.

DC B04

IN JACQUEMIN, M G; SAINT-REMY, J R

PA (COLL-N) COLLEN RES FOUND VZW D; (JACQ-I) JACQUEMIN M G; (SAIN-I) SAINT-REMY J R

CYC 27

PI EP 1222929 A2 20020717 (200266)* EN 41 A61K039-395
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
US 2003175268 A1 20030918 (200382)# 29 A61K039-395

ADT EP 1222929 A2 EP 2002-447005 20020111; US 2003175268 A1 US 2002-44569 20020111

PRAI US 2001-261405P 20010111; US 2002-44569 20020111

IC ICM A61K039-395
ICS A61P007-02

ICA C07K016-36

ICI A61K031:727, A61K039-395

AB EP 1222929 A UPAB: 20040102
NOVELTY - A pharmaceutical composition (I) for the prevention and/or treatment of systemic inflammatory response syndrome (SIRS) and/or sepsis and/or septic shock and/or thrombus formation in the microvasculature and/or disseminated intravascular coagulation in mammals, comprising as an active ingredient a partial inhibitor (II) of factor VIII, in admixture with a carrier.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for the use of (II) for the manufacture of a medicine for the prevention and/or treatment of SIRS and/or sepsis and/or septic shock and/or thrombus formation in the microvasculature and/or disseminated intravascular

coagulation in mammals.

ACTIVITY - Antibacterial; Immunosuppressive; Antiinflammatory.

MECHANISM OF ACTION - Partial inhibitor of factor VIII; partial inhibitor of formation of thrombin.

(II) (A human monoclonal antibody obtainable from the cell line named KRIX 1 deposited with the Belgian coordinated collections of microorganisms under accession number LMBP 5089CB) was tested in vitro against the rate of thrombin formation using whole blood. Citrated whole blood was recalcified and shaken at 900 rpm at 37 deg. C in 24-well cell suspension culture plates avoiding contact activation. Samples were then transferred at regular intervals to a solution containing ethylenediaminetetraacetic acid (EDTA) in order to stop further coagulation activation. Following centrifugation, thrombin formation was then determined by measuring optical density using the chromogenic substrate S-2238. Results showed that the addition of 2.5 micro g/ml of human monoclonal antibodies derived from the cell line KRIX 1 significantly reduced (by about 1/3) but did not abolish the rate of thrombin formation as compared to the control.

USE - (I) Is useful for the prevention and/or treatment of systemic inflammatory response syndrome (SIRS) and/or sepsis and/or septic shock and/or thrombus formation in the microvasculature and/or disseminated intravascular coagulation in mammals (claimed), and also useful for restoring plasma level of anti-thrombin and/or activated protein C and/or **tissue factor pathway inhibitor** in mammals. (I) Is useful for treating and/or preventing blood circulation disorders following a systemic infection with gram negative microorganisms in a mammal, preferably a human.

ADVANTAGE - The partial inhibitors of factor VIII efficiently but only partially inhibit the co-factor activity of factor VIII, even when used in a more than 100-fold excess, i.e. they achieve a plateau effect in the inactivation of factor VIII, thereby avoiding the risk of overdosing the patient. The human IgG antibodies (i.e. (II)) exhibit a prolonged half-life time of three weeks (except for IgG3 which is one week), thus providing very stable plasma levels of the therapeutically active agent and allowing for a drastic reduction in the frequency of administration. Further, the use of human antibodies, fragments or homologues thereof, carries a minimal risk of inducing an immune response in the patient. (II) Inhibits the function of fVIII to an extent sufficient to reduce, or partially inhibit, the formation of thrombin. Reduction, but not complete suppression, of the formation of thrombin prevents the development of disseminated intravascular coagulation (DIC) while allowing normal clot formation. Preventing DIC maintains normal organ perfusion and avoids organ dysfunction and failure. (II) Keeps the formation of thrombin under control and thus reduces activation of the compensatory anti-inflammatory response. Thus, activated protein C is generated by direct thrombin cleavage and the effect of **tissue factor pathway inhibitor** is dependent on the presence of activated factor X, the activation of which is directly dependent on factor VIII co-factor activity. Since the antibodies used in this invention only partially neutralize factor VIII, there is no inherent risk of spontaneous bleeding.

Dwg.0/13

FS CPI

FA AB; DCN

MC CPI: B04-C02E1; B04-F0100E; B04-G2100E; B04-N0200E; B14-A01; B14-C03; B14-F04; B14-J07

L81 ANSWER 18 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AN 2003:165666 BIOSIS

DN PREV200300165666

TI **Purification**, Molecular Cloning and Expression of A Novel Growth Promotive Factor for Retinal Pigment Epithelial Cells, REF-1/**TFPI**-2.

AU Tanaka, Y. [Reprint Author]; Utsumi, J.; Sudo, T.; Nakamura, N.; Kigasawa, K.; Iwata, T. [Reprint Author]; Matsui, M.

CS National Center for Sensory Organs, National Tokyo Medical Center, Tokyo, Japan

SO ARVO Annual Meeting Abstract Search and Program Planner, (2002) Vol. 2002, pp. Abstract No. 4583. cd-rom.
Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology. Fort Lauderdale, Florida, USA. May 05-10, 2002.

DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LA English

ED Entered STN: 2 Apr 2003
Last Updated on STN: 2 Apr 2003

AB Purpose: Retinal pigment epithelial (RPE) cells are known to play important roles to maintain homeostasis of the retina and to control choroidal neovascularization. The factor which stimulates RPE cell growth should be very valuable for treatment of some retinal diseases. The purpose of this study is to discover a specific growth promotive factor for RPE cells. **Methods**: One hundred liter of human fibroblast conditioned medium was applied to ion-exchange, hydrophobic and reversed-phase chromatographies and sodium dodecyl sulphate-polyacrylamide gel electrophoresis to **purify** RPE cell growth promotive activity. Human K-1034 RPE cells for the activity determination were originally established as reported earlier (Kigasawa et al.Japan J. Ophthalmol. 38, 10-15, 1994). Human endothelial cells were separated from human umbilical veins. Cell growth activity was determined by cultivation of test cells with samples from chromatographies. Molecular cloning was performed from placental cDNA library. The expression vector of cloned cDNA of the growth promotive factor was constructed with pSRalphavector and transfected to Chinese ovary (CHO) cells. The expressed recombinant protein was **purified** and characterized. Results: We found and isolated 31kDa factor that has growth promotive activity for RPE cells but not for human endothelial cells (REF-1: RPE cell factor-1). The amino-terminal sequence of REF-1 was identical to that of tissue-factor pathway inhibitor-2 (**TFPI**-2), a family of **TFPIs** or placental protein 5 (PP5), a serine protease inhibitor. The cDNA of REF-1/**TFPI**-2 was cloned from placental cDNA library and expressed in CHO cells. Recombinant REF-1/**TFPI**-2 was 31kDa as a major component and expressed a growth promotive activity for RPE cells but not for endothelial cells and has protease inhibitory activity in vitro. The other family factor, **TFPI**-1 did not promote RPE cell growth. Conclusion: We found a novel activity of **TFPI**-2 as a growth promotive factor for RPE cells, REF-1. Because REF-1/**TFPI**-2 did not stimulate endothelial cells, the factor may have beneficial effect for the repair and maintenance of RPE cells membrane in vivo.

CC General biology - Symposia, transactions and proceedings 00520
Cytology - General 02502
Cytology - Animal 02506
Cytology - Human 02508
Biochemistry studies - General 10060
Sense organs - Physiology and biochemistry 20004

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Sense Organs (Sensory Reception)

IT Chemicals & Biochemicals
growth promotive factor: expression, molecular cloning, **purification**

IT Methods & Equipment
molecular cloning: genetic techniques, laboratory techniques
ORGN Classifier
Cricetidae 86310
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
CHO cell line (cell line): Chinese hamster ovary cells
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
K-1034 cell line (cell line): human retinal pigment epithelial cells
REF-1 cell line (cell line): human retinal epithelial cells
TFPI-2 cell line (cell line): human retinal epithelial cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

L81 ANSWER 20 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2002:55552 BIOSIS
DN PREV200200055552
TI **Method** of **solubilizing**, **purifying**, and refolding protein.
AU Dorin, Glenn J. [Inventor]; Arve, Bo H. [Inventor]; Pattison, Gregory L. [Inventor]; Halenbeck, Robert F. [Inventor, Reprint author]; Johnson, Kirk [Inventor]; Chen, Bao-Lu [Inventor]; Rana, Rajsharan K. [Inventor]; Hoba, Maninder S. [Inventor]; Madani, Hassan [Inventor]; Tsang, Michael [Inventor]; Gustafson, Mark E. [Inventor]; Bild, Gary S. [Inventor]; Johnson, Gary V. [Inventor]
CS San Rafael, CA, USA
ASSIGNEE: Chiron Corporation; G. D. Searle and Co.
PI US 6323326 November 27, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 27, 2001) Vol. 1252, No. 4. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 9 Jan 2002
Last Updated on STN: 25 Feb 2002
AB A **method** of modifying protein **solubility** employs polyionic polymers. These facilitate the **solubilization**, **formulation**, **purification** and refolding of proteins especially incorrectly folded proteins and aggregated proteins. Compositions are described that are suitable for **formulating** **TFPI**. The compositions allow preparation of pharmaceutically acceptable compositions of **TFPI** at concentrations above 0.2 mg/mL and above 10 mg/mL.
NCL 530412000
CC Biochemistry studies - General 10060
Biochemistry studies - Proteins, peptides and amino acids 10064
IT Major Concepts
Biochemistry and Molecular Biophysics; **Methods** and Techniques
IT Chemicals & Biochemicals
protein
IT Methods & Equipment

method of **solubilizing**, **purifying**, and refolding protein: biochemical **method**, **purification method**

L81 ANSWER 21 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:71770 BIOSIS
DN PREV200200071770
TI **Method** of **solubilizing**, **purifying**, and refolding protein.
AU Dorin, Glenn J. [Inventor, Reprint author]; Arve, Bo H. [Inventor]; Pattison, Gregory L. [Inventor]; Halenbeck, Robert F. [Inventor]; Johnson, Kirk [Inventor]; Chen, Bao-Lu [Inventor]; Rana, Rajsharan K. [Inventor]; Hora, Maninder S. [Inventor]; Madani, Hassan [Inventor]; Tsang, Michael [Inventor]; Gustafson, Mark E. [Inventor]; Bild, Gary S. [Inventor]; Johnson, Gary V. [Inventor]
CS San Rafael, CA, USA
ASSIGNEE: Chiron Corporation; G.D. Searle and Co.
PI US 6319896 November 20, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 20, 2001) Vol. 1252, No. 3. ftp://ftp.uspto.gov/pub/patdata/. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English
ED Entered STN: 16 Jan 2002
Last Updated on STN: 25 Feb 2002
AB A **method** of modifying protein **solubility** employs polyionic polymers. These facilitate the **solubilization**, **formulation**, **purification** and refolding of proteins especially incorrectly folded proteins and aggregated proteins. Compositions are described that are suitable for **formulating TFPI**. The compositions allow preparation of pharmaceutically acceptable compositions of **TFPI** at concentrations above 0.2 mg/mL and above 10 mg/mL.
NCL 514008000
CC Enzymes - General and comparative studies: coenzymes 10802
Pathology - Therapy 12512
Pharmacology - General 22002
IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); **Methods** and Techniques; Pharmacology
IT Chemicals & Biochemicals
polyionic polymers
IT Methods & Equipment
protein modification: modification **method**

L81 ANSWER 22 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:366080 BIOSIS
DN PREV200100366080
TI Heterocyclic compounds regulating clotting.
AU Persson, Egon [Inventor, Reprint author]; Jakobsen, Palle [Inventor]; Worsaae, Helle [Inventor]
CS Malmphi, Sweden
ASSIGNEE: Novo Nordisk A/S, Bagsvaerd, Denmark
PI US 6180625 January 30, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 30, 2001) Vol. 1242, No. 5. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DT Patent
LA English

ED Entered STN: 2 Aug 2001
Last Updated on STN: 19 Feb 2002
AB Compounds of **formula** (I) ##STR1## as factor VII-**tissue factor inhibitors** as well as novel benzoxazin derivatives are disclosed, wherein R1, R2, R3, X and Y are as defined in the specification. These compounds, and pharmaceutically acceptable salts thereof, have been shown to be inhibitors of factor VIIa-tissue factor activity and have anticoagulant properties. These compounds are useful for treating deficiencies of blood clotting factors or the effects of inhibitors to blood clotting factors. **Methods** for inhibiting clotting activity are also disclosed.
NCL 514230500
CC General biology - Miscellaneous 00532
IT Major Concepts
Hematology (Human Medicine, Medical Sciences); Pharmacology
IT Chemicals & Biochemicals
heterocyclic clot regulating compounds: anticoagulant-drug

L81 ANSWER 23 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2001-514662 [56] WPIX
DNC C2001-153853
TI Protein C derivative for treating acute coronary syndromes, vascular occlusive disorders, thrombotic disorders and sepsis, comprises substitutions at specified amino acid positions.
DC B04 D16
IN GERLITZ, B E; GRINNELL, B W; JONES, B E
PA (ELIL) LILLY & CO ELI; (GERL-I) GERLITZ B E; (GRIN-I) GRINNELL B W; (JONE-I) JONES B E
CYC 95
PI WO 2001059084 A1 20010816 (200156)* EN 59 C12N009-64
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001032799 A 20010820 (200175) C12N009-64
EP 1263943 A1 20021211 (200301) EN C12N009-64
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
US 2003022354 A1 20030130 (200311) C12N009-64
JP 2003521938 W 20030722 (200350) 55 C12N015-09
US 6630138 B2 20031007 (200374) C12N009-64
ADT WO 2001059084 A1 WO 2001-US1221 20010202; AU 2001032799 A AU 2001-32799 20010202; EP 1263943 A1 EP 2001-904860 20010202, WO 2001-US1221 20010202; US 2003022354 A1 WO 2001-US1221 20010202, US 2002-182263 20020722; JP 2003521938 W JP 2001-558224 20010202, WO 2001-US1221 20010202; US 6630138 B2 Provisional US 2000-181948P 20000211, Provisional US 2000-189199P 20000314, WO 2001-US1221 20010202, US 2002-182263 20020722
FDT AU 2001032799 A Based on WO 2001059084; EP 1263943 A1 Based on WO 2001059084; JP 2003521938 W Based on WO 2001059084; US 6630138 B2 Based on WO 2001059084
PRAI US 2000-189199P 20000314; US 2000-181948P 20000211; US 2002-182263 20020722
IC ICM C12N009-64; C12N015-09
ICS A61K038-48; A61K045-00; A61K048-00; A61P007-02; A61P009-10; A61P031-04; A61P043-00; C07H021-04; C12N001-15; C12N001-19; C12N001-20; C12N001-21; C12N005-06; C12N005-10; C12N009-00; C12N015-00; C12N015-85; C12P021-02
AB WO 200159084 A UPAB: 20011001

NOVELTY - A human protein C derivative (I) comprising a sequence of 419 amino acids fully defined in the specification, where amino acid Asp at position 167 is substituted with Phe and at position 172 is substituted with Lys, is new.

DETAILED DESCRIPTION - A new human protein C derivative (I) comprises a sequence of 419 amino acids fully defined in the specification, where amino acid Asp at position 167 is substituted with Phe and at position 172 is substituted with Lys. (I) further comprises at least one amino acid substitution selected from His at position 10 and Ser at positions 11 and 12 are independently substituted with any amino acid, Gln at position 32 is substituted with Glu, Asn at position 33 is substituted with Asp or Phe, and, amino acids at positions 194, 195, 228, 249, 254, 302, or 316 are substituted with Ser, Ala, Thr, His, Leu, Lys, Arg, Asn, Asp, Glu, Gly and Gln.

INDEPENDENT CLAIMS are also included for the following:

(1) a recombinant DNA molecule (II) encoding (I);

(2) a pharmaceutical composition (PC) comprising (I);

(3) a vector (III) comprising (II);

(4) a host cell (IV) transformed by (III); and

(5) producing (I).

ACTIVITY - Thrombolytic; anticoagulant; antibacterial; immunosuppressive; antianemic; cardiant; antianginal; vulnerary; antisickling; virucide; hemostatic.

MECHANISM OF ACTION - Gene therapy. No supporting data is given.

USE - (I) is useful for treating acute coronary syndromes and disease states predisposing to thrombosis, vascular occlusive disorders and hypercoagulable states, sepsis in combination with bactericidal permeability increasing protein or **tissue factor pathway inhibitor**, thrombotic disorders in combination with an anti-platelet agent, protein C deficiency and acute arterial thrombotic occlusion, thromboembolism or stenosis in coronary cerebral or peripheral arteries or in vascular grafts in combination with a thrombolytic agent. DNA (II) encoding (I) is useful for treating human patients with genetically predisposed prothrombotic disorders by gene therapy. (I) is also useful in the manufacture of a medicament for the treatment of acute coronary syndromes, vascular occlusive disorders and hypercoagulable states, sepsis in combination with bactericidal permeability increasing protein or **tissue factor pathway inhibitor**, thrombotic disorders in combination with an anti-platelet agent and genetically predisposed prothrombotic disorders (claimed). (I) is useful for treating acute coronary syndromes e.g. myocardial infarction and unstable angina, and vascular occlusive disorders and hypercoagulable states e.g. sepsis, disseminated intravascular coagulation (DIC), burns, transplantations, thalassemia, sickle cell disease, viral hemorrhagic fever and hemolytic uremic syndrome.

ADVANTAGE - (I) has increased anticoagulation activity, resistance to serpin inactivation and increased sensitivity to thrombin activation compared to wild type protein C, and retains the biological activity of the wild type human protein C. The derivatives will require either less frequent administration and/or smaller dosages than wild type human protein C in the treatment of acute coronary syndromes, vascular occlusive disorder, hypercoagulable states and thrombotic disorders.

Dwg.0/0

FS CPI

FA AB; DCN

MC CPI: B04-E02F; B04-E03F; B04-E08; B04-F0100E; B04-N04A; B04-N04A0E; B11-B; B14-A01; B14-A02; B14-E10; B14-F01; B14-F02; B14-F03; B14-F04; B14-G02; B14-G02C; B14-N17A; B14-N17B; B14-S03; D05-C12; D05-H12B; D05-H12E; D05-H14; D05-H17B6

L81 ANSWER 26 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 2001-308197 [32] WPIX
DNC C2001-095201
TI New combination of an amino acid base buffered by an acid free of its salt form increases the stability of polypeptides in pharmaceutical compositions whilst increasing isotonicity to reduce pain during administration.
DC B04
IN CHEN, B; HORA, M; HORA, M S
PA (CHIR) CHIRON CORP
CYC 95
PI WO 2001024814 A1 20010412 (200132)* EN 84 A61K038-20
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000078475 A 20010510 (200143) A61K038-20
NO 2002001567 A 20020522 (200247) A61K000-00
EP 1220682 A1 20020710 (200253) EN A61K038-20
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI
BR 2000014486 A 20020917 (200264) A61K038-20
CZ 2002001186 A3 20021113 (200282) A61K038-20
HU 2002003133 A2 20021228 (200308) A61K038-20
JP 2003510368 W 20030318 (200321) 91 A61K038-00
US 6525102 B1 20030225 (200323) A01N025-00
CN 1402640 A 20030312 (200339) A61K038-20
US 2003180253 A1 20030925 (200364) A61K038-20
ADT WO 2001024814 A1 WO 2000-US27156 20001003; AU 2000078475 A AU 2000-78475 20001003; NO 2002001567 A WO 2000-US27156 20001003, NO 2002-1567 20020403; EP 1220682 A1 EP 2000-968584 20001003, WO 2000-US27156 20001003; BR 2000014486 A BR 2000-14486 20001003, WO 2000-US27156 20001003; CZ 2002001186 A3 WO 2000-US27156 20001003, CZ 2002-1186 20001003; HU 2002003133 A2 WO 2000-US27156 20001003, HU 2002-3133 20001003; JP 2003510368 W WO 2000-US27156 20001003, JP 2001-527813 20001003; US 6525102 B1 Provisional US 1999-157696P 19991004, US 2000-677643 20001003; CN 1402640 A CN 2000-816328 20001003; US 2003180253 A1 Provisional US 1999-157696P 19991004, Cont of US 2000-677643 20001003, US 2002-299039 20021118
FDT AU 2000078475 A Based on WO 2001024814; EP 1220682 A1 Based on WO 2001024814; BR 2000014486 A Based on WO 2001024814; CZ 2002001186 A3 Based on WO 2001024814; HU 2002003133 A2 Based on WO 2001024814; JP 2003510368 W Based on WO 2001024814; US 2003180253 A1 Cont of US 6525102
PRAI US 1999-157696P 19991004; US 2000-677643 20001003; US 2002-299039 20021118
IC ICM A01N025-00; A61K000-00; A61K038-00; A61K038-20
ICS A61K009-08; A61K009-20; A61K038-17; A61K038-21; A61K038-57; A61K039-00; A61K045-00; A61K047-04; A61K047-12; A61K047-18; A61P035-00; A61P043-00; C07K001-02; C07K017-00
AB WO 200124814 A UPAB: 20021031
NOVELTY - Stabilized liquid pharmaceutical composition (I) comprising a polypeptide or its variant, an amino acid base comprising arginine, lysine, aspartic acid or glutamic acid and a buffering agent.
DETAILED DESCRIPTION - New stabilized liquid pharmaceutical composition (I) comprises:
(a) an active agent consisting polypeptide (or one of its variants), exhibiting aggregate formation during storage in a liquid

formulation;

(b) an amino acid base present in an amount sufficient to decrease aggregate formation of the polypeptide or its variant during storage of the composition, the amino acid base comprising at least one amino acid selected from arginine, lysine, aspartic acid and glutamic acid; and

(c) a buffering agent selected from an acid free of its salt forms and/or an acid in its salt form.

INDEPENDENT CLAIMS are also included for:

(1) a stabilized liquid pharmaceutical composition comprising interleukin-2 (IL-2) or one of its variants, arginine in its free base form (150-350 mM) and succinic acid substantially free of its salt form (80-190 mM);

(2) a stabilized liquid pharmaceutical composition comprising **tissue factor pathway inhibitor** (TFPI) or one of its variants, arginine in its free base form (175-325 mM) and succinic acid substantially free of its salt form (80-190 mM);

(3) a stabilized liquid pharmaceutical composition comprising **tissue factor pathway inhibitor** (TFPI) or one of its variants, arginine in its free base form (175-400 mM) and citric acid substantially free of its salt form (40-200 mM); and

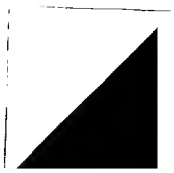
(4) a method for increasing the stability of a polypeptide or one of its variants in a liquid pharmaceutical composition, where the polypeptide or it variant exhibits aggregate formation during storage in a liquid **formulation**, by incorporating into the composition an amino acid base in an amount sufficient to decrease aggregate formation of the polypeptide or one of its variants and a buffer selected from acids free of their salt forms, acids in their salt forms and a mixture of an acids and its salt form, the amino acid base comprising at least one amino acid selected form arginine, lysine, aspartic acid and glutamic acid.

USE - The invention is for stabilizing polypeptides such as IL-2 (used in the treatment of cancer metastasis) in pharmaceutical compositions.

ADVANTAGE - The increased storage stability of the composition is achieved through the influence of the amino acid on the stability of the therapeutically active polypeptide, in particular through its influence on polypeptide aggregation during storage in liquid **formulations.** The incorporation of an amino acid base and an acid free of its salt form within liquid polypeptide-containing **formulations** results in liquid pharmaceutical compositions that are near isotonic without having to include additional isotonizing agents. Isotonicity reduces pain upon administration and the compositions of the invention exhibit reduced pain associated with burning and stinging relative to injection of normal saline. The novel combination of the invention allows for **formulations** with higher concentrations of the stabilizing amino acid than can be achieved with the use of a buffer system that is a mixture of an acid and its salt form. The higher concentration of the stabilizing amino acid allows for even greater increases in polypeptide stability, and thus increased storage stability of the **formulation** . In a stability study, **tissue factor pathway inhibitor** was **formulated** in 0.15 mg/ml final concentration in various **formulations** containing either L-arginine base or L-arginine hydrochloride. L-arginine hydrochloride **formulations** were buffered to pH 5.5 by citric acid or succinic acid (10 mM) in combination with its respective conjugate salt. L-arginine base **formulation** was titrated to pH 5.5 by either citric or succinic acid. It was found that acid titration with either succinic or citric acid allowed for a greater concentration of arginine in the **formulation** while maintaining isotonicity. However one of these **formulations** using 10 mM citric acid and sodium citrate to buffer 300 mM L-arginine hydrochloride to pH 5.5. had a solution osmolarity of

497 mOsm/kg which constitutes a hypertonic **formulation** which is not preferable for injection. On the other hand, a **formulation** using 121 mM citric acid in combination with 300 mM L-arginine base to adjust the pH to 5.5 had a solution osmolarity of 295 mOsm/kg, which is very close to an isotonic solution and preferable for injection.
Dwg.0/11
FS CPI
FA AB; DCN
MC CPI: B04-C01; B04-H02B; B04-H02B0E; B04-N04; B04-N0400E; B10-A17; B10-A18; B10-B02J; B10-C02; B14-L06

L81 ANSWER 36 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
AN 2000:489754 BIOSIS
DN PREV200000489875
TI Prokaryotic expression, **purification**, and reconstitution of biological activities (antiprotease, antitumor, and heparin-binding) for tissue factor pathway inhibitor-2.
AU Rao, C. N. [Reprint author]; Reddy, Prasad; Reeder, Dennis J.; Liu, Yueying; Stack, Sharon M.; Kisiel, W.; Woodley, David T.
CS Center for Prostate Disease Research, 1530 East Jefferson Street, Rockville, MD, 20852, USA
SO Biochemical and Biophysical Research Communications, (October 5, 2000) Vol. 276, No. 3, pp. 1286-1294. print.
CODEN: BBRCA9. ISSN: 0006-291X.
DT Article
LA English
ED Entered STN: 15 Nov 2000
Last Updated on STN: 10 Jan 2002
AB We report the expression of tissue factor pathway inhibitor-2 (**TFPI**-2) (also known as PP-5, placental protein-5; MSPI, matrix-associated serine protease inhibitor) in E. coli as a 25-kDa nonglycosylated protein with a glycine substituted for aspartic acid at the amino terminus. High-level expression of **TFPI**-2 was obtained with pRE1 expression vector under the transcriptional and translational controls of the lambdaPL promoter and lambdacII ribosome-binding site, respectively, with ATG initiation codon. **TFPI**-2 was produced as inclusion bodies and accounted for 25-30% of the total E. coli proteins. The inclusion bodies containing **TFPI**-2 were **solubilized** with urea, sulfitolyzed, **purified**, and refolded through a disulfide interchange reaction. The refolded E. coli **TFPI**-2 inhibited plasmin with an inhibition constant (Ki) of 5 nM that is similar with the **TFPI**-2 expressed in a mammalian system. The refolded E. coli **TFPI**-2 bound heparin and also inhibited plasmin, regardless of whether the enzyme was in the fluid phase or was bound to the membranes of HT-1080 fibrosarcoma cells. In addition, refolded E. coli **TFPI**-2 inhibited radiolabeled matrix degradation and Matrigel matrix invasion by HT-1080 fibrosarcoma cells and B16-F10 melanoma cells. Together, our results suggest that glycosylation is not essential for antiprotease, antitumor, and matrix-binding activities of **TFPI**-2. Based on these collective data, we conclude that a biologically active nonglycosylated **TFPI**-2 can be produced in E. coli and that the protein can be produced in high-enough quantities to conduct in vivo studies for determination of the role of this inhibitor in tumor invasion and metastasis.
CC Physiology and biochemistry of bacteria 31000
Cytology - Animal 02506
Cytology - Human 02508
Biochemistry studies - General 10060



Biochemistry studies - Carbohydrates 10068
Enzymes - General and comparative studies: coenzymes 10802
Neoplasms - Pathology, clinical aspects and systemic effects 24004
IT Major Concepts
Biochemistry and Molecular Biophysics; **Methods** and Techniques
IT Diseases
fibrosarcoma: neoplastic disease
Fibrosarcoma (MeSH)
IT Diseases
melanoma: neoplastic disease
Melanoma (MeSH)
IT Chemicals & Biochemicals
heparin; pRE1: expression vector; plasmin: inhibition; tissue factor pathway inhibitor-2: antiprotease, antitumor protein, glycosylation, **purification**, synthesis
IT Methods & Equipment
bacterial expression system: synthetic **method**
ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
E. coli [Escherichia coli]
Taxa Notes
Bacteria, Eubacteria, Microorganisms
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HT-1080 cell line
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
B16-F10 cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates
RN 9005-49-6 (heparin)
9001-90-5 (plasmin)
160477-63-4 (tissue factor pathway inhibitor-2)

L81 ANSWER 40 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1999-288168 [24] WPIX
CR 1998-531707 [45]
DNC C1999-085199
TI Peptide composition.
DC B04 D16
IN CINES, D; HIGAZI, A A
PA (UYPE-N) UNIV PENNSYLVANIA
CYC 23
PI WO 9920295 A1 19990429 (199924)* EN 62 A61K038-08
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP US
AU 9913609 A 19990510 (199938) A61K038-08

EP 1030679 A1 20000830 (200042) EN A61K038-08
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
AU 739373 B 20011011 (200171) A61K038-08
JP 2001520200 W 20011030 (200202) 68 A61K038-00
US 6750201 B1 20040615 (200439) A61K038-08

ADT WO 9920295 A1 WO 1998-US21800 19981015; AU 9913609 A AU 1999-13609 19981015; EP 1030679 A1 EP 1998-957325 19981015, WO 1998-US21800 19981015; AU 739373 B AU 1999-13609 19981015; JP 2001520200 W WO 1998-US21800 19981015, JP 2000-516692 19981015; US 6750201 B1 Provisional US 1997-62274P 19971017, Cont of WO 1998-US21800 19981015, US 2000-544665 20000406

FDT AU 9913609 A Based on WO 9920295; EP 1030679 A1 Based on WO 9920295; AU 739373 B Previous Publ. AU 9913609, Based on WO 9920295; JP 2001520200 W Based on WO 9920295

PRAI US 1997-62274P 19971017; US 2000-544665 20000406

IC ICM A61K038-00; A61K038-08
ICS A61K038-10; A61K038-16; A61K038-48; A61K038-55; A61K045-00; A61P009-00; A61P009-10; A61P015-00; A61P035-00; A61P035-04; A61P043-00; C07K007-06

AB WO 9920295 A UPAB: 19990624

NOVELTY - Peptide composition that can be used to affect a biological process characterized by abnormal cell migration through a physiological barrier, to inhibit PAI-1-dependent adhesion of a cell to a tissue, to promote clearance of scuPA from the surface of a mammalian cell, and to impede pathological migration of a cell in a mammal.

DETAILED DESCRIPTION - A composition (A) comprising a peptide having the amino acid sequence (I).

X1 = hydrogen, an amino-terminal blocking group or one to twenty amino acid residues;

X2 = an amino acid selected from the group consisting of D, E, H, K, and R;

X3 = an amino acid selected from the group consisting of E and D;

X4 = an amino acid selected from the group consisting of I, L, and V;

X5 = an amino acid selected from the group consisting of I. L, and V;

X6 = an amino acid selected from the group consisting of M;

X7 = an amino acid selected from the group consisting of D, E, H, K, and R; and

X8 = hydrogen, a carboxyl-terminal blocking group, or one to twenty amino acid residues.

INDEPENDENT CLAIMS are also included for the following:

(1) a method of affecting a biological process characterized by abnormal cell migration through a physiological barrier, the method comprises administering (A) to a mammal experiencing the biological process in an amount to affect the biological process;

(2) a method of inhibiting PAI-1-dependent adhesion of a cell to a tissue of a mammal, the method comprising administering (A) to the tissue in an amount effective to inhibit adhesion of the cell to the tissue;

(3) a method of promoting clearance of scuPA from the surface of a mammalian cell, comprising administering (A) in an amount to promote clearance of the scuPA from the cell;

(4) a method of impeding pathological migration of a cell in a mammal, the method comprising administering (A) in an amount effective to impede pathological migration of the cell;

(5) a method of inhibiting PAI-1 activity in a tissue of a mammal, comprising administering (A) to the tissue in an amount effective to inhibit PAI-1 activity in the tissue;

(6) a kit comprising a peptide having an amino acid sequence of **formula** (I), and an instructional material for using the kit; and

(7) a composition comprising a combination of a peptide having an amino acid sequence of **formula** (I), and a thrombolytic or an

anti-coagulating agent.

ACTIVITY - Cytostatic; antiinflammatory; antiarteriosclerotic.

MECHANISM OF ACTION - None given.

USE - The composition can be used to affect a biological process characterized by abnormal cell migration through a physiological barrier, where the process is selected from angiogenesis, organogenesis, ovulation, inflammation, cancer, tumor cell invasion and metastasis, and atherosclerosis. It can also be used to inhibit PAI-1-dependent adhesion of a cell to a tissue, to promote clearance of scuPA from the surface of a mammalian cell, and to impede pathological migration of a cell in a mammal (all claimed).

ADVANTAGE - None given.

FS CPI
FA AB; GI; DCN
MC CPI: B04-C01B; B04-C01F; B14-C03; B14-F07; B14-H01; D05-A02

L81 ANSWER 41 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1999-120504 [10] WPIX
DNC C1999-035197
TI A new vector comprising a human **tissue factor pathway inhibitor** - useful to provide an antithrombotic agent to vascular smooth muscle cells.
DC B04 D16
IN WILLERSON, J T; ZOLDHELYI, P
PA (TEXA-N) TEXAS HEART INST
CYC 83
PI WO 9902171 A1 19990121 (199910)* EN 28 A61K035-76
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW
AU 9879546 A 19990208 (199924) A61K035-76
EP 1001795 A1 20000524 (200030) EN A61K035-76
R: CH DE ES FR GB IT LI
US 6214333 B1 20010410 (200122) A61K048-00
JP 2001509367 W 20010724 (200147) 56 C12N015-09
ADT WO 9902171 A1 WO 1998-US11706 19980605; AU 9879546 A AU 1998-79546 19980605; EP 1001795 A1 EP 1998-930076 19980605, WO 1998-US11706 19980605; US 6214333 B1 Provisional US 1997-51887P 19970708, US 1998-13366 19980126; JP 2001509367 W WO 1998-US11706 19980605, JP 2000-501761 19980605
FDT AU 9879546 A Based on WO 9902171; EP 1001795 A1 Based on WO 9902171; JP 2001509367 W Based on WO 9902171
PRAI US 1998-13366 19980126; US 1997-51887P 19970708
IC ICM A61K048-00; C12N015-09
ICS A61P007-02; A61P009-10; C12N005-10; C12N007-02; C12N015-63; C12N015-64; C12N015-86; C12N015-861
ICA A61K035-76; A61K038-00
AB WO 9902171 A UPAB: 19990310

A new recombinant adenoviral vector (V1) comprises a human **tissue factor pathway inhibitor** (TFPI) gene operably linked to a human cytomegalovirus immediate early (CMV) promoter/enhancer and a simian leukaemia virus (SV40) polyadenylation site.

Also claimed are:

(1) a recombinant adenoviral vector comprising Ad.TFPI;

(2) transduced vascular smooth muscle cells comprising a transgenic TFPI;

(3) a method of making a recombinant adenovirus, comprising: (a) ligating a cDNA encoding human full length TFPI into the BamHI site of the

polylinker in pACCMVpLpA plasmid to form the pLpA.TFPI shuttle plasmid; (b) co-transfecting mammalian cells in tissue culture with the shuttle plasmid and plasmid pJM17; (c) culturing the transfected cells until viral cytopathic effects appear; (d) harvesting Ad.TFPI viral stock from the culture; (e) inoculating monolayers of mammalian cells in tissue culture with high titre Ad.TFPI viral stock; (f) harvesting culture medium and cells on appearance of cytopathic effects; (g) **purifying** recombinant virion particles containing Ad.TFPI;

(4) a method of making an antithrombotic agent, by obtaining virion particles according to the above method, and using them to infect vascular smooth muscle (VSM) cells so that the cells express TFPI cDNA

(5) a method of transducing a VSM cell, particularly a human VSM cell, by introducing into it V1;

(6) a method of producing human TFPI at a predetermined site in a blood vessel by exposing VSM cells at the site to V1;

(7) a method of maintaining a therapeutic level of human TFPI at a predetermined site by transfecting at least 106 VSM cells at the site to V1 so that the TFPI gene is expressed for at least 3 days.

USE - The invention is used to deter thrombosis deposition at a blood vessel site, particularly where the site is a balloon catheter injured artery site, an atherosclerotic artery site, an angioplasty site, an arteriovenous shunt, or an endovascular graft (claimed). The invention is also used to deter development of chronic vascular stenosis in blood vessels (arteries, veins, arteriovenous shunts and endovascular grafts) and to deter intimal hyperplasia.

ADVANTAGE - The invention provides antithrombotic treatment without the risk of increased haemorrhage and requirement for hospitalisation associated with prior art systemically administered drugs.

Dwg.0/8

FS CPI
FA AB
MC CPI: B04-E02B; B14-F04; B14-S03; D05-H09; D05-H12E; D05-H14B2

L81 ANSWER 43 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:164993 BIOSIS
DN PREV199900164993
TI Tissue factor pathway inhibitor-2 is a novel mitogen for vascular smooth muscle cells.
AU Shinoda, Eiji; Yui, Yoshiki [Reprint author]; Hattori, Ryuichi; Tanaka, Misaki; Inoue, Reiko; Aoyama, Takeshi; Takimoto, Yoshihito; Mitsui, Youji; Miyahara, Kaoru; Shizuta, Yutaka; Sasayama, Shigetake
CS 26-4 Maehagicho, Shimogamo, Sakyo-Ku, Kyoto City 6060833, Japan
SO Journal of Biological Chemistry, (Feb. 26, 1999) Vol. 274, No. 9, pp. 5379-5384. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DT Article
LA English
ED Entered STN: 16 Apr 1999
Last Updated on STN: 16 Apr 1999
AB A mitogen for growth-arrested cultured bovine aortic smooth muscle cells was **purified** to homogeneity from the supernatant of cultured human umbilical vein endothelial cells by heparin affinity chromatography and reverse-phase high performance liquid chromatography. This mitogen was revealed to be tissue factor pathway inhibitory (**TFPI**-2), which is a Kunitz-type serine protease inhibitor. **TFPI**-2 was expressed in baby hamster kidney cells using a mammalian expression vector. Recombinant **TFPI**-2 (rTFPI-2) stimulated DNA synthesis and cell proliferation in a dose-dependent manner (1-500 nM). rTFPI-2 activated mitogen-activated protein kinase (MAPK) activity and stimulated early proto-oncogene c-fos mRNA expression in smooth muscle cells. MAPK,

c-fos expression and the mitogenic activity were inhibited by a specific inhibitor of MAPK kinase, PD098059. Thus, the mitogenic function of rTFPI-2 is considered to be mediated through MAPK pathway. **TFPI** has been reported to exhibit antiproliferative action after vascular smooth muscle injury in addition to the ability to inhibit activation of the extrinsic coagulation cascade. However, structurally similar **TFPI**-2 was found to have a mitogenic activity for the smooth muscle cell.

CC Biochemistry studies - Proteins, peptides and amino acids 10064
Methods - Laboratory methods 01004
Cytology - Human 02508
Biochemistry methods - Proteins, peptides and amino acids 10054
Biophysics - Molecular properties and macromolecules 10506
Enzymes - General and comparative studies: coenzymes 10802
Cardiovascular system - Physiology and biochemistry 14504
General biology - Miscellaneous 00532

IT Major Concepts
Biochemistry and Molecular Biophysics; Cardiovascular System (Transport and Circulation); Cell Biology; **Methods** and Techniques

IT Parts, Structures, & Systems of Organisms
vascular smooth muscle cells: circulatory system, muscular system

IT Chemicals & Biochemicals
tissue factor pathway inhibitor-2: Kunitz-type serine protease inhibitor, **purification**, novel mitogen, identification; c-fos gene: expression, proto-oncogene

IT Methods & Equipment
c-fos promoter luciferase reporter assay: activity assays, analytical **method**; heparin affinity chromatography: affinity chromatography, **purification method**; mitogen-activated protein kinase activity assay: activity assays, analytical **method**; mitogen-activated protein kinase phosphorylation: chemical modification, chemical modification **method**; reverse phase high performance liquid chromatography: high performance liquid chromatography, **purification method**; tissue factor pathway inhibitor-2 cDNA expression vector construction [tissue factor pathway inhibitor-2 complementary DNA expression vector construction]: Recombinant DNA Technology, genetic engineering **method**; transfection: gene expression/vector techniques, genetic engineering **method**; Bio-Rad protein assay: Bio-Rad, analytical **method**, protein concentration assays; HiTrap Heparin affinity column: Amersham Pharmacia Biotech, laboratory equipment; LC-6A high performance liquid chromatography system: Shimadzu Co., laboratory equipment; MAP kinase assay kit: New England Biolabs, laboratory equipment; Northern blot: Recombinant DNA Technology, molecular probe techniques, gene mapping, analytical **method**, detection/labeling techniques; Phast System: Amersham Pharmacia Biotech, laboratory equipment; SDS-polyacrylamide gel electrophoresis: polyacrylamide gel electrophoresis, **purification method**

ORGN Classifier
Cricetidae 86310
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
BHK cell line
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

ORGN Classifier
Hominidae 86215

Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
HUVEC cell line
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 160477-63-4 (tissue factor pathway inhibitor-2)
9003-05-8 (POLYACRYLAMIDE)
9005-49-6 (HEPARIN)
9014-00-0 (LUCIFERASE)
9026-43-1 (PROTEIN KINASE)
9031-44-1 (KINASE)

L81 ANSWER 44 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:488384 BIOSIS
DN PREV199900488384
TI Coexpression of tissue factor and tissue factor pathway inhibitor by human monocytes **purified** by leukapheresis and elutriation. Response of nonadherent cells to lipopolysaccharide.
AU Nguyen, P. [Reprint author]; Broussas, M.; Cornillet-Lefebvre, P.; Potron, G.
CS Laboratoire central d'Hematologie, Avenue du General Koenig, 51092, Reims Cedex, France
SO Transfusion (Bethesda), (Sept., 1999) Vol. 39, No. 9, pp. 975-982. print.
CODEN: TRANAT. ISSN: 0041-1132.
DT Article
LA English
ED Entered STN: 16 Nov 1999
Last Updated on STN: 5 Jun 2000
AB BACKGROUND: Counterflow centrifugal elutriation is the **method** of choice for obtaining a large quantity of highly **purified** monocytes. In spite of the fact that this technique has been used for many years to isolate monocytes for cellular immunotherapy, it is not known whether the process of elutriation can stimulate tissue factor (TF) expression and therefore trigger coagulation in patients receiving these cell preparations. The aim of the present study is thus to identify TF and TF pathway inhibitor (**TFPI**) in elutriated monocytes and to evaluate their ability to trigger thrombin generation. STUDY DESIGN AND **METHODS**: Human monocytes are separated by leukapheresis and elutriation in sterile, endotoxin-free conditions. TF and **TFPI** mRNA is detected by reverse transcription-polymerase chain reaction. TF and **TFPI** are measured by enzyme-linked immunosorbent assay in cell lysates. TF antigen expression on cell surface is evidenced by direct-flow cytometry. Two functional tests (a chronometrictest and an amidolytic assay) assess the capacity of monocytes to trigger thrombin generation. The response to lipopolysaccharide (LPS) is evaluated with each technique. Monocytic cell line THP-1 is used as a positive control. RESULTS: Elutriated monocytes coexpress TF mRNA and **TFPI** mRNA. The expression of TF mRNA is dramatically increased by LPS activation. This is correlated with a 100-fold increase in the amount of TF antigen in monocyte lysates. Flow immunocytometry confirms the expression of TF antigen on cell membrane in response to LPS stimulation, whereas **TFPI** mRNA is slightly increased after LPS stimulation. The amount of **TFPI** antigen in cell lysates is small when compared to that in plasma. Elutriated monocytes have a strong potential to trigger thrombin generation in response to LPS. CONCLUSION: In spite of the coexpression of TF mRNA and **TFPI** mRNA, elutriated monocytes are capable of supporting prothrombinase activity. This should be taken into account in the evaluation ofthe safety of adoptive cellular immunotherapy.
CC Blood - General and methods 15001

Cytology - Human 02508
Immunology - General and methods 34502
Enzymes - General and comparative studies: coenzymes 10802
IT Major Concepts
Hematology (Human Medicine, Medical Sciences)
IT Parts, Structures, & Systems of Organisms
monocyte: blood and lymphatics, immune system
IT Chemicals & Biochemicals
lipopolysaccharide: activation; mRNA [messenger RNA]; prothrombinase; thrombin: generation; tissue factor; tissue factor pathway inhibitor
IT Methods & Equipment
cellular immunotherapy: therapeutic **method**; counterflow centrifugal elutriation: collection **method**; leukapheresis: cytapheresis, separation **method**
ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human: patient
THP-1 cell line: monocyte cell
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 72162-96-0 (prothrombinase)
9002-04-4 (thrombin)
194554-71-7 (tissue factor pathway inhibitor)

L81 ANSWER 45 OF 67 IPA COPYRIGHT 2004 ASHP on STN DUPLICATE 4

AN 2000:13711 IPA
DN 37-13712
TI Solubility of recombinant human tissue factor pathway inhibitor
AU Chen, B. L.; Wu, X.; Babuka, S. J.; Hora, M.
CS Dept. of Formulation Development, Chiron Corp., 4560 Horton St., Emeryville, CA 94608-2916, USA Internet: bao-lu_chen@cc.chiron.com
SO Journal of Pharmaceutical Sciences (USA), (Sep 1999) Vol. 88, pp. 881-888. 24 Refs.
CODEN: JPMSAE; ISSN: 0022-3549.
DT Journal
LA English
AB The solubility of recombinant human tissue factor pathway inhibitor (rhTFPI) in various solvent systems at 4DGC and at ambient temperature, the effect of pH (range 3-11) and ionic strength at pH 5, 6, and 7 on the solubility of rhTFPI, and resolubilization of insolubility precipitates of rhTFPI formed due to limited protein solubility and instability precipitates of rhTFPI resulting from protein instability were studied.

The results showed that under various solvent conditions, the solubility of rhTFPI was predominantly affected by the charge distribution on the protein molecule itself as well as on the solvent ions present in the surrounding medium. The solubility of rhTFPI at 4DGC was slightly higher than at ambient temperature, independent of the type of solvent. rhTFPI showed minimum solubility between pH 5 and 10. A third solubilizing phase for rhTFPI at pH 5 under low ionic strength conditions that was different from the normal salting in and salting out phases of the protein was identified. Also, differences in the resolubilization of insolubility and instability precipitates of rhTFPI were observed.
Ramune T. Dailide
SC 9 Pharmaceutics; 10 Drug Stability
CC 20:12.04 Anticoagulants
IT Tissue factor pathway inhibitor; solubility; analysis

IT Anticoagulants; tissue factor pathway inhibitor; solubility
IT **Solubility; tissue factor pathway inhibitor; analysis**
IT Solvents; tissue factor pathway inhibitor; solubility
IT Temperature; tissue factor pathway inhibitor; solubility
IT Hydrogen ion concentration; tissue factor pathway inhibitor; solubility
IT Ionic strength; tissue factor pathway inhibitor; solubility
IT Precipitates; tissue factor pathway inhibitor; solubility
IT Charge; tissue factor pathway inhibitor; solubility
RN **194554-71-7 (Tissue factor pathway inhibitor)**

L81 ANSWER 46 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1999:164810 BIOSIS
DN PREV199900164810
TI TFPIbeta, a second product from the mouse tissue factor pathway inhibitor (**TFPI**) gene.
AU Chang, Jen-Yea [Reprint author]; Monroe, Dougald M.; Oliver, Julie A.; Roberts, Harold R.
CS 932 Mary Ellen Jones Build., CB 7035, Univ. N.C., Chapel Hill, NC 27599-7035, USA
SO Thrombosis and Haemostasis, (Jan., 1999) Vol. 81, No. 1, pp. 45-49. print. CODEN: THHADQ. ISSN: 0340-6245.
DT Article
LA English
ED Entered STN: 16 Apr 1999
Last Updated on STN: 16 Apr 1999
AB Tissue factor pathway inhibitor (**TFPI**) contains three Kunitz domains separated by two connecting regions. We have cloned another naturally occurring **TFPI** gene product from a mouse lung cDNA library which we have called TFPIbeta. TFPIbeta is derived from alternative splicing of the **TFPI** gene. Analysis of the cDNA shows that mouse TFPIbeta protein is identical to **TFPI** from the N'-terminus through the second connecting region. However, mouse TFPIbeta possesses neither a third Kunitz domain nor an Arg, Lys-rich C'-terminus but instead has a completely different C'-terminal (beta-domain) sequence which is not homologous to any known protein. Northern blot analyses show that the tissues for mouse TFPIbeta synthesis are heart and lung; in contrast, **TFPI** appears in Northern blots of heart and spleen. Both TFPIbeta and **TFPI** messages first appear in 7-day-old mouse embryos, but only the **TFPI** mRNA persists until 17 days. **Purified** recombinant TFPIbeta shows an apparent molecular weight of 38 kDa. Kinetic studies indicate that mouse TFPIbeta is a slow-binding enzyme inhibitor for human factor Xa. In addition, heparin does not enhance the inhibition of factor Xa by mouse TFPIbeta although it does accelerate factor Xa inhibition by **TFPI.**
CC Blood - General and methods 15001
Genetics - Animal 03506
Biochemistry studies - General 10060
Biophysics - General 10502
IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Molecular Genetics (Biochemistry and Molecular Biophysics)
IT Chemicals & Biochemicals
TFBI-beta: complementary DNA library, gene product, heart, protein homology, spleen, molecular cloning, lung; mouse tissue factor pathway inhibitor gene [mouse **TFPI** gene]: alternative splicing
IT Methods & Equipment
Northern blot analysis: analytical **method**
IT Miscellaneous Descriptors
amino acid sequence; nucleotide sequence
ORGN Classifier

Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse: embryo
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

L81 ANSWER 47 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1998-437473 [37] WPIX
DNC C1998-133106
TI Isolated **tissue factor pathway inhibitor**-3 - used to develop products for treating, e.g. pulmonary embolism, thrombosis, sepsis, inflammatory disease, transplant rejection or haemophilia.
DC B04 D16
IN GENTZ, R L; HSU, T; NI, J; ROSEN, C A
PA (HUMA-N) HUMAN GENOME SCI INC
CYC 82
PI WO 9833920 A2 19980806 (199837)* EN 57 C12N015-15
RW: AT BE CH DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW
AU 9860422 A 19980825 (199903) C12N015-15
EP 1005551 A2 20000607 (200032) EN C12N015-15
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 2001504709 W 20010410 (200128) 97 C12N015-09
US 6262233 B1 20010717 (200142) C07K004-00
US 2001029034 A1 20011011 (200162) C07H021-04
US 6548262 B2 20030415 (200329) G01N033-50
US 2003187200 A1 20031002 (200365) C07K014-435
ADT WO 9833920 A2 WO 1998-US1468 19980127; AU 9860422 A AU 1998-60422 19980127; EP 1005551 A2 EP 1998-903730 19980127, WO 1998-US1468 19980127; JP 2001504709 W JP 1998-532993 19980127, WO 1998-US1468 19980127; US 6262233 B1 Provisional US 1997-36703P 19970131, US 1998-13896 19980127; US 2001029034 A1 Provisional US 1997-36703P 19970131, Div ex US 1998-13896 19980127, US 2001-827948 20010406; US 6548262 B2 Provisional US 1997-36703P 19970131, Div ex US 1998-13896 19980127, US 2001-827948 20010406; US 2003187200 A1 Provisional US 1997-36703P 19970131, Div ex US 1998-13896 19980127, Div ex US 2001-827948 20010409, US 2002-176071 20020621
FDT AU 9860422 A Based on WO 9833920; EP 1005551 A2 Based on WO 9833920; JP 2001504709 W Based on WO 9833920; US 2001029034 A1 Div ex US 6262233; US 6548262 B2 Div ex US 6262233; US 2003187200 A1 Div ex US 6262233, Div ex US 6548262
PRAI US 1997-36703P 19970131; US 1998-13896 19980127; US 2001-827948 20010406; US 2002-176071 20020621
IC ICM C07H021-04; C07K004-00; C07K014-435; C12N015-09; C12N015-15; G01N033-50
ICS A61K031-705; A61K038-00; A61K038-57; A61K039-395; A61K045-00; A61P007-04; A61P009-00; C07K014-81; C07K016-38; C12N001-15; C12N001-19; C12N001-21; C12N005-00; C12N005-02; C12N005-10; C12N015-00; C12N015-11; C12N015-63; C12N015-70; C12N015-74; C12P021-02; C12P021-06; G01N033-48
AB WO 9833920 A UPAB: 19981021
An isolated nucleic acid molecule (NAM) (A) comprises a polynucleotide

(PN) having a nucleotide sequence (NS) at least 95% identical to a sequence selected from:

(a) a NS encoding a polypeptide comprising the predicted second Kunitz-type domain of the **tissue factor pathway inhibitor**-3 (TFPI-3) polypeptide having an amino acid sequence at positions 106 to 156 of a sequence given in the specification or as encoded by a cDNA clone contained in ATCC 97797;

(b) a NS encoding a polypeptide comprising the complete consensus Kunitz-type domain having an amino acid sequence as given in the specification, and

(c) a NS complementary to any of the NSs in (a) or (b) where the NS of (a) and (b) does not encode a polypeptide comprising an amino acid sequence selected from the sequence of **formula** (I)-(III):

Ala Asp Arg Glu Arg Ser Ile His Asp Phe Xaa Leu Val Ser Lys (I);

Lys Val Val Gly Arg Xaa Arg Ala Ser Met Pro Arg Trp Trp Tyr Asn Val Thr Asp Gly Ser Xaa Gln Leu Phe Val Tyr Gly Gly (II), and

Ala Thr Val Thr Glu Asn Ala Thr Gly Asp Leu Ala Thr Ser Arg Asn Ala Ala Asp Ser Ser Val Pro Ser Ala Pro (III).

Also claimed are:

(1) an isolated NAM comprising a PN having a NS at least 95% identical to a sequence selected from:

(a) a NS encoding a polypeptide comprising an amino acid sequence of residues n-225 of a sequence given in the specification, where n is an integer in the range of 99-106;

(b) a NS encoding a polypeptide comprising an amino acid sequence of residues 99-m of a sequence given in the specification, where m is an integer in the range of 156-225;

(c) a NS encoding a polypeptide having an amino acid sequence consisting of residues n-m of a sequence given in the specification, where n and m are integers as in (a) and (b);

(d) a NS encoding a polypeptide consisting of a portion of the complete TFPI-3 amino acid sequence encoded by a cDNA clone contained in ATCC 97797, where the portion excludes from 126 to 132 amino acids from the amino acid terminus of the complete amino acid sequence encoded by a cDNA clone contained in ATCC 97797;

(e) a NS encoding a polypeptide consisting of a portion of the complete TFPI-3 amino acid sequence encoded by a cDNA clone contained in ATCC 97797 where the portion excludes from 1 to 69 amino acids from the carboxy terminus of the complete amino acid sequence encoded by a cDNA contained in ATCC 97797, and

(f) a NS encoding a polypeptide consisting of a portion of the complete TFPI-3 amino acid sequence encoded by a cDNA clone contained in ATCC 97797 where the portion includes a combination of any of the amino terminal and carboxy terminal deletions in (d) and (e);

(2) a method for making a recombinant vector comprising inserting an isolated NAM as in (A) into a vector;

(3) a recombinant vector produced by a method as in (2);

(4) a method of making a recombinant host cell comprising introducing the recombinant vector as in (3) into a host cell;

(5) a recombinant host cell produced by a method as in (4);

(6) an isolated TFPI-3 polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from:

(a) an amino acid sequence of a polypeptide comprising the complete consensus Kunitz-type domain having an amino acid sequence (given in the specification), and

(b) an amino acid sequence of a polypeptide comprising the second Kunitz-type domain of TFPI-3- having an amino acid sequence at positions 106 to 156 of a sequence given in the specification; or as encoded by the cDNA clone contained in ATCC 97797, and

(7) an antagonist against a polypeptide as in (6).

USE - The TFPI-3 inhibits protease activity. TFPI-3 polypeptides can be used for, e.g. inhibiting intravascular clotting and preventing the formation of fibrin clots both in vitro and in vivo, for anticoagulant therapy in prophylaxis of venous thrombosis and as treatment for preventing its extension, as well as to provide low-dose regiment for prevention of post-operative deep venous thrombosis and pulmonary embolism, for the prophylaxis and treatment of pulmonary embolism and atrial fibrillation with embolism, to prevent clotting in arterial and heart surgery as well as for prevention of cerebral thrombosis in evolving stroke, for treating coronary occlusion with acute myocardial infarction and in the prophylaxis and treatment of peripheral arterial embolism, for the treatment of sepsis, inflammatory diseases, transplant rejection, in the treatment of hyperfibrinolytic haemorrhage and traumatic haemorrhagic shock as well as in diseases connected with excessive release of pancreatic elastase (pancreatitis), serum elastase (artherosclerosis), leukocyte elastase in acute and chronic inflammation with damage to connective tissue, in damage to vessel walls, in necrotic diseases, and in degeneration of lung tissue. TFPI-3 can also be used as an anticoagulant in blood transfusions, extracorporeal circulation, and dialysis procedures and in blood samples for laboratory purposes The antagonists can be used to promote coagulation, e.g. in the treatment of haemophilia. The products can also be used for detection, diagnosis and drug screening.

Dwg.0/5

FS CPI

FA AB

MC CPI: B04-E02F; B04-E08; B04-F01; B04-N04; B12-K04; B14-C03; B14-F04; B14-G02C; B14-K01; B14-S07; D05-H09; D05-H12A; D05-H12E; D05-H14; D05-H17A

L81 ANSWER 50 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

AN 1999:26542 BIOSIS

DN PREV199900026542

TI Preparation and characterization of monoclonal antibody against recombinant human tissue factor pathway inhibitor.

AU Yang, Yinke; He, Xiaofan; Li, Juncheng; He, Shilin

CS Dep. Physiol., Hunan Med. Univ., Changsha 410078, China

SO Chinese Medical Journal (English Edition), (Aug., 1998) Vol. 111, No. 8, pp. 718-721. print.
CODEN: CMJODS. ISSN: 0366-6999.

DT Article

LA English

ED Entered STN: 20 Jan 1999
Last Updated on STN: 20 Jan 1999

AB Objective: To prepare and identify monoclonal antibody (McAb) against recombinant human tissue factor pathway inhibitor (rhTFPI) and to use it for measurement of **TFPI** by ELISA, and to evaluate the effects of the McAb on dilute prothrombin time (PT) and activated partial thromboplastin time (APTT). **Methods**: After intrasplenic immunization of Balb/c mouse with **TFPI**, hybridoma technique was used to raise monoclonal antibody against rhTFPI. The McAb was well-characterized and labelled with horseradish peroxidase (HRP) by using assay of **TFPI** in ELISA. Furthermore, the McAb was added to normal and factor IX deficient plasma for observation of dilute PT and APTT. Results: Two hybridomas (4F4, 4F8) secreting McAb against **TFPI** were established. Ig class and subclass of the McAb **purified** from 4F8 was IgG1. Immunoblotting results indicated that the McAb4F8 only recognized a single band of **TFPI** with molecular weight of 34.8 KD. The results of Sandwich enzyme-linked immunosorbent assay (ELISA) by using the HRP labelled McAb4F8 showed that the mean of **TFPI** in normal human plasma is 103.2 +- 11.5 mug/L. The McAb 4F8

was also proved to shorten markedly dilute prothrombin time of factor IX deficient plasma and normal plasma. Conclusions: We established two hybridomas cell lines (4F4, 4F8) and obtained the McAb4F8 against **TFPI** and reported the levels of **TFPI** in healthy adult human plasma by Sandwich ELISA with HRP labelled McAb4F8 in Chinese.

CC Blood - General and methods 15001
Biochemistry studies - General 10060
Biophysics - General 10502
Enzymes - General and comparative studies: coenzymes 10802
Immunology - General and methods 34502

IT Major Concepts
Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Immune System (Chemical Coordination and Homeostasis)

IT Chemicals & Biochemicals
horseradish peroxidase: label; monoclonal antibody: characterization, preparation; recombinant human tissue factor pathway inhibitor: anticoagulant protein

IT Methods & Equipment
ELISA: measurement **method**

IT Miscellaneous Descriptors
activated partial thromboplastin time; prothrombin time

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
BALB/c mouse
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN 9001-26-7 (PROTHROMBIN)
9002-05-5Q (THROMBOPLASTIN)
9003-99-0 (PEROXIDASE)
9035-58-9Q (THROMBOPLASTIN)
72162-96-0Q (THROMBOPLASTIN)

L81 ANSWER 51 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:131801 BIOSIS
DN PREV199800131801
TI Cloning, expression, and characterization of mouse tissue factor pathway inhibitor (**TFPI**).
AU Chang, Jen-Yea [Reprint author]; Monroe, Dougald M.; Oliver, Julie A.; Liles, Darla K.; Roberts, Harold R.
CS 932 Mary Ellen Jones Building CB 7035, University North Carolina Chapel Hill, Chapel Hill, NC 27599-7035, USA
SO Thrombosis and Haemostasis, (Feb., 1998) Vol. 79, No. 2, pp. 306-309. print.
CODEN: THHADQ. ISSN: 0340-6245.
DT Article
LA English
OS Genbank-AF004833
ED Entered STN: 20 Mar 1998

Last Updated on STN: 20 Mar 1998

AB Tissue factor pathway inhibitor (**TFPI**) acts to regulate the initiation of coagulation by first inhibiting factor Xa. The complex of factor Xa/ **TFPI** then inhibits the factor VIIa/tissue factor complex. The cDNA sequences of **TFPI** from several different species have been previously reported. A high level of similarity is present among **TFPIs** at the molecular level (DNA and protein sequences) as well as in biochemical function (inhibition of factor Xa, VIIa/tissue factor). In this report, we used a PCR-based screening **method** to clone cDNA for full length **TFPI** from a mouse macrophage cDNA library. Both cDNA and predicted protein sequences show significant homology to the other reported **TFPI** sequences, especially to that of rat. Mouse **TFPI** has a signal peptide of 28 amino acid residues followed by the mature protein (in which the signal peptide is removed) which has 278 amino acid residues. Mouse **TFPI** , like that of other species, consists of three tandem Kunitz type domains. Recombinant mouse **TFPI** was expressed in the human kidney cell line 293 and **purified** for functional assays. When using human clotting factors to investigate the inhibition spectrum of mouse **TFPI**, it was shown that, in addition to human factor Xa, mouse **TFPI** inhibits human factors VIIa, IXa, as well as factor XIa. Cloning and expression of the mouse **TFPI** gene will offer useful information and material for coagulation studies performed in a mouse model system.

CC Cardiovascular system - General and methods 14501
Biochemistry studies - General 10060
Blood - General and methods 15001
Immunology - General and methods 34502

IT Major Concepts
Cardiovascular System (Transport and Circulation)

IT Parts, Structures, & Systems of Organisms
macrophages: blood and lymphatics, immune system

IT Chemicals & Biochemicals
tissue factor pathway inhibitor [**TFPI**]: expression

IT Sequence Data
AF004833: Genbank, nucleotide sequence

IT Miscellaneous Descriptors
cloning

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
293: human kidney cells
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

ORGN Classifier
Muridae 86375
Super Taxa
Rodentia; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
mouse: animal model
Taxa Notes
Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates

RN **194554-71-7** (tissue factor pathway inhibitor)
194554-71-7 (**TFPI**)

L81 ANSWER 54 OF 67 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 5
AN 1997:107451 HCAPLUS

DN 126:122446
ED Entered STN: 15 Feb 1997
TI Method of **solubilizing**, purifying, and refolding protein
IN Dorin, Glenn J.; Arve, Bo H.; Pattison, Gregory L.; Hallenbeck, Robert F.; Johnson, Kirk; Chen, Bao-Lu; Rana, Rajsharan K.; Hora, Maninder S.; Madani, Hassan; Gustafson, Mark E.; Tsang, Michael; Bild, Gary S.; Johnson, Gary V.
PA Chiron Corporation, USA; G.D. Searle and Co.; Dorin, Glenn J.; Arve, Bo H.; Pattison, Gregory L.; Hallenbeck, Robert F.; Johnson, Kirk; Chen, Bao-Lu; Rana, Rajsharan, K.; et al.
SO PCT Int. Appl., 84 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C07K014-81
ICS C07K001-113; A61K038-57; A61K009-08; A61K047-30
CC 63-3 (Pharmaceuticals)
FAN.CNT 12

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9640784	A2	19961219	WO 1996-US9980	19960607
	WO 9640784	A3	19970313		
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM				
	CA 2223745	AA	19961219	CA 1996-2223745	19960607
	AU 9664770	A1	19961230	AU 1996-64770	19960607
	AU 713338	B2	19991202		
	EP 837883	A2	19980429	EP 1996-924269	19960607
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	JP 11514334	T2	19991207	JP 1996-502126	19960607
	US 5888968	A	19990330	US 1996-734997	19961022
	US 6323326	B1	20011127	US 1999-973211	19990611
	US 6319896	B1	20011120	US 1999-443098	19991118
	AU 759412	B2	20030417	AU 2000-20611	20000302
	US 2002137884	A1	20020926	US 2001-996588	20011130
	JP 2004083591	A2	20040318	JP 2003-326585	20030918
PRAI	US 1995-473668	A	19950607		
	US 1995-477677	A	19950607		
	US 1995-473688	B1	19950607		
	AU 1996-64770	A3	19960607		
	JP 1997-502126	A3	19960607		
	WO 1996-US9980	W	19960607		
	US 1999-973211	A3	19990611		
	US 1999-443099	B1	19991118		

AB A method of modifying protein **solubility** employs polyionic polymers. These facilitate the **solubilization**, formulation, purification and refolding of proteins especially incorrectly folded proteins and aggregated proteins. Compns. are described that are suitable for formulating tissue factor pathway inhibitor (TFPI). The compns. allow preparation of pharmaceutically acceptable composition of TFPI at concns. above 0.2 mg/mL and above 10 mg/mL.
ST protein refolding purifn **solubilization**; **TFPI** refolding purifn **solubilization**; tissue factor pathway inhibitor refolding
IT **Solubilizers**

(**solubilizing**, purifying, and refolding proteins)
IT Polyoxyalkylenes, biological studies
Proteins, general, biological studies
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**solubilizing**, purifying, and refolding proteins)
IT Polysaccharides, biological studies
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(sulfated; **solubilizing**, purifying, and refolding proteins)
IT **194554-71-7P**
RL: PEP (Physical, engineering or chemical process); PRP (Properties); PUR (Purification or recovery); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); PROC (Process); USES (Uses)
(**solubilizing**, purifying, and refolding proteins)
IT 57-50-1, Sucrose, biological studies 25322-68-3
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**solubilizing**, purifying, and refolding proteins)
IT 50-70-4, D-Glucitol, biological studies 56-40-6, Glycine, biological studies 69-65-8, D-Mannitol 71-00-1, Histidine, biological studies 127-09-3, Sodium acetate 151-21-3, Sodium dodecyl sulfate, biological studies 288-32-4, Imidazole, biological studies 320-77-4 994-36-5, Sodium citrate 7632-05-5, Sodium phosphate 7647-14-5, Sodium chloride (NaCl), biological studies 9005-49-6, Heparin, biological studies 9042-14-2, Dextran sulfate 11070-68-1, Glutamate, biological studies 14047-56-4
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**solubilizing**, purifying, and refolding proteins)

L81 ANSWER 56 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1996-129393 [13] WPIX
DNC C1996-040392
TI Production of **tissue factor pathway inhibitor** in yeast cells - with isolation from the **insoluble** cell fraction, used to treat or prevent sepsis or septic shock.
DC B04 D16
IN CREASEY, A A; INNIS, M A
PA (CHIR) CHIRON CORP
CYC 22
PI WO 9604377 A1 19960215 (199613)* EN 36 C12N015-15
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA JP MX
AU 9531044 A 19960304 (199623) C12N015-15
EP 774001 A1 19970521 (199725) EN C12N015-15
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 10507071 W 19980714 (199838) 38 C12N015-09
AU 707762 B 19990722 (199940) C12N015-15
US 6103500 A 20000815 (200041) C12N015-00
EP 774001 B1 20021016 (200276) EN C12N015-15
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
DE 69528591 E 20021205 (200304) C12N015-15
CA 2196296 C 20040406 (200425) EN C07K014-81
ADT WO 9604377 A1 WO 1995-US9377 19950725; AU 9531044 A AU 1995-31044 19950725; EP 774001 A1 EP 1995-926779 19950725, WO 1995-US9377 19950725; JP 10507071 W WO 1995-US9377 19950725, JP 1996-506588 19950725; AU 707762 B AU 1995-31044 19950725; US 6103500 A Cont of US 1994-286530 19940805, US 1997-854764 19970512; EP 774001 B1 EP 1995-926779 19950725, WO 1995-US9377

19950725; DE 69528591 E DE 1995-628591 19950725, EP 1995-926779 19950725, WO 1995-US9377 19950725; CA 2196296 C CA 1995-2196296 19950725, WO 1995-US9377 19950725
FDT AU 9531044 A Based on WO 9604377; EP 774001 A1 Based on WO 9604377; JP 10507071 W Based on WO 9604377; AU 707762 B Previous Publ. AU 9531044, Based on WO 9604377; EP 774001 B1 Based on WO 9604377; DE 69528591 E Based on EP 774001, Based on WO 9604377; CA 2196296 C Based on WO 9604377
PRAI US 1994-286530 19940805; US 1997-854764 19970512
REP 04Jnl.Ref
IC ICM C07K014-81; C12N015-00; C12N015-09; C12N015-15
ICS C07K019-00; C12N015-81; C12P021-02; C12P021-06
ICA C12N001-19
ICI C12N001-19, C12R001:865
AB WO 9604377 A UPAB: 19960329
Production of a factor VIIa/TF (tissue factor)/Xa binding protein (I, i.e. a **tissue factor pathway inhibitor**, TFPI) comprises: (1) incubating yeast cells transformed with a replicable cloning vehicle containing a sequence encoding (I), such that (I) is retained within the cells; (2) preparing an **insoluble** fraction of the cells, containing (I), and; (3) isolating (I) from the **insoluble** fraction. Also claimed is (I) produced by the above method.
USE - (I) inhibits the coagulation pathway and may be useful for treating or preventing sepsis or septic shock.
ADVANTAGE - This method produces full length, homogeneous (I) with the correct N-terminal sequence.
Dwg.0/4
FS CPI
FA AB
MC CPI: B04-E02F; B04-F09C0E; B14-A01; B14-J07; D05-H17A6; D05-H17C

L81 ANSWER 57 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1995-280847 [37] WPIX
DNN N1995-214145 DNC C1995-126635
TI Thrombus formation inhibitor for treating vascular anastomosis - comprises **tissue factor pathway inhibitor** and PEG.
DC A96 B04 D16 D22 P34
PA (KAGA) ZH KAGAKU & KESSEI RYOHO KENKYUSHO
CYC 1
PI JP 07179360 A 19950718 (199537)* 6 A61K038-55
ADT JP 07179360 A JP 1994-298962 19941107
PRAI JP 1993-304640 19931109
IC ICM A61K038-55
ICS A61K047-32; A61L033-00
ICA A61L029-00
AB JP 07179360 A UPAB: 19950921
Inhibitor of thrombus formation comprises **tissue factor pathway inhibitor** (TFPI) and PEG.
The PEG has a mol. weight of 4000-6000. PEG is melted at 40 deg.C in boiled water, mixed with a TFPI solution, filled in a catheter and solidified by cooling to form a PEG stick impregnated with TFPI. Alternatively, a PEG stick without TFPI is formed similarly and then spread with TFPI. TFPI is prepared by gene technology. cDNA of TFPI is connected to a shuttle vector containing chicken beta-actin promoter to form a plasmid for TFPI expression. The plasmid is introduced in Chinese hamster ovary cells, which are cultured in a medium containing polysaccharide sulphate to induce a cell line with high productivity of TFPI. TFPI produced by the recombinant cells may be separated from the medium by ultrafiltration followed by **purificn** . using affinity chromatography.
USE/ADVANTAGE - The drug compsn. inhibits blockage of blood vessels

by thrombus. It is especially effective for vascular anastomosis of severely damaged blood vessels. Thrombus formation in vascular anastomosis of blood vessels severely damaged by crush or compression, may be prevented by application of the drug compsn., especially a solid **formulation** of PEG impregnated or spread with TPFI (both claimed), inside the surgically connected vessel. By using PEG the inhibiting effect of TPFI on thrombus formation may be sustained.

In an example, 2g of reagent-grade PEG 4000 melted in boiled water was mixed with TFPI dissolved in an aqueous solution containing 20 mM sodium citrate and 0.15M NaCl (pH 7.4) so that the TFPI concentration was 20 microgram/ml PEG. The melt was introduced in a catheter and solidified by cooling. The PEG stick was taken off and **formulated** to a size fitted inside a blood vessel. A thigh artery of an anaesthetised Wistar rat was pinched with a haemostat and was given a severe fracture of a needle, followed by cutting-off. The PEG stick was inserted into both ends of a cut vessel and the vessel was surgically connected, and the haemostat was removed. After 15 mins. normal blood flow was observed in the operated vessel, and no thrombus was found in 9 out of 10 rats operated. Rats receiving the operation but not treated with the PEG-TFPI caused total blockage of blood flow by thrombus without exception.

Dwg.0/0

FS CPI GMPI
FA AB; DCN
MC CPI: A05-H03; A12-V01; A12-V03B; B04-C03C; B14-F04; B14-L06; D05-H12E; D05-H13

L81 ANSWER 60 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1994-333111 [41] WPIX
DNC C1994-151546
TI **Purificn.** of factor VII with controlled activation and degradation - used in preparation of activated highly pure blood coagulation factor.
DC B04
IN JORGENSEN, T; PEDERSEN, A H; PEDERSEN, A; JOERGENSEN, T
PA (NOVO) NOVO-NORDISK AS; (CARO-N) CAROMA IND LTD
CYC 31
PI WO 9422905 A1 19941013 (199441)* EN 24 C07K003-22
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU CA CN CZ FI HU JP KR NO PL RU US
ZA 9401956 A 19941130 (199503) 21 C07K000-00
AU 9464239 A 19941024 (199505) C07K003-22
FI 9504649 A 19950929 (199550) C07K000-00
NO 9503883 A 19951128 (199606) C07K001-16
EP 691984 A1 19960117 (199608) EN C07K003-22
R: AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE
CZ 9502533 A3 19960117 (199610) C07K014-745
TW 278079 A 19960611 (199639) C07K003-22
JP 08508264 W 19960903 (199704) 21 C07K014-745
AU 677309 B 19970417 (199723) C07K003-28
HU 72712 T 19960528 (199743) C07K001-16
CN 1121723 A 19960501 (199745) C07K001-16
US 5700914 A 19971223 (199806) 8 C07K003-22
ADT WO 9422905 A1 WO 1994-DK122 19940324; ZA 9401956 A ZA 1994-1956 19940321; AU 9464239 A AU 1994-64239 19940324; FI 9504649 A WO 1994-DK122 19940324, FI 1995-4649 19950929; NO 9503883 A WO 1994-DK122 19940324, NO 1995-3883 19950929; EP 691984 A1 EP 1994-911854 19940324, WO 1994-DK122 19940324; CZ 9502533 A3 CZ 1995-2533 19940324; TW 278079 A TW 1994-102182 19940314; JP 08508264 W JP 1994-521552 19940324, WO 1994-DK122 19940324; AU 677309 B AU 1994-64239 19940324; HU 72712 T WO 1994-DK122 19940324, HU 1995-2846

19940324; CN 1121723 A CN 1994-191833 19940324; US 5700914 A WO 1994-DK122 19940324, US 1995-446671 19950528
FDT AU 9464239 A Based on WO 9422905; EP 691984 A1 Based on WO 9422905; JP 08508264 W Based on WO 9422905; AU 677309 B Previous Publ. AU 9464239, Based on WO 9422905; HU 72712 T Based on WO 9422905; US 5700914 A Based on WO 9422905
PRAI DK 1993-382 19930331
REP 02Jnl.Ref; **EP** 363126
IC ICM C07K000-00; C07K001-16; C07K003-22; C07K003-28; C07K014-745
ICS C07K001-18; C07K001-22; C07K001-36
ICA A61K035-12; A61K035-16; A61K038-36; A61K038-43
AB WO 9422905 A UPAB: 19941206
Controlled activation and degradation of factor VII during **purificn.** comprises chromatographic **purificn.** where Zn2+ is present in 21 of the steps.

USE/ADVANTAGE - Coagulation factor VII is a vitamin K dependent serine protease playing a key role in the **extrinsic** pathway of **blood coagulation.** In its activated form factor VII (factor VIIa) catalyses the activation of two other vitamin K dependent coagulation factors, factor IX and factor X. Factor VIIa may be used in treating patients who have developed inhibitors to factor VIII and for the treatment of patients suffering from bleeding disorders such as platelet disorders including thrombocytopoemia, von Willebrand's disease and others typically present in association with severe tissue damage. In contrast to factor VIIa the single chain form is resistant to or much less prone to cleavage in the heavy chain and it is advantageous to **purify** factor VII in its single chain form. The process allows this whilst avoiding or maintaining activation and degradation at an acceptable low degree and gives a homogeneous prod. of high purity which can then be activated into factor VIIa in high yields to give a uniform and homogeneous prod. with high specific activity.

Dwg.0/3
FS CPI
FA AB
MC CPI: B04-H19; B05-A03A; B14-F08

L81 ANSWER 62 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 1994:205342 BIOSIS
DN PREV199497218342
TI Refold and characterization of recombinant tissue-factor pathway inhibitor expressed in Escherichia coli.
AU Diaz-Collier, Judy A.; Palmier, Mark O.; Kretzmer, Kuniko K.; Bishop, Bruce F.; Combs, Rodney G.; Obukowicz, Mark G.; Frazier, Ronald B.; Bild, Gary S.; Joy, William D.
CS Monsanto Co. AA4E, 700 Chesterfield Parkway, Chesterfield, MO 63198, USA
SO Thrombosis and Haemostasis, (1994) Vol. 71, No. 3, pp. 339-346.
CODEN: THHADQ. ISSN: 0340-6245.
DT Article
LA English
ED Entered STN: 10 May 1994
Last Updated on STN: 10 May 1994
AB Human tissue factor pathway inhibitor (**TFPI**) was expressed in E. coli as a non-glycosylated protein with an additional alanine attached to the aminoterminus of the wild type molecule. High-level expression was obtained with pMON6875, a plasmid containing a tac promoter, Gene 10 leader from bacteriophage T7, methionine-alanine-**TFPI** coding sequence, and the p22 transcriptional terminator. In this system, **TFPI** accounted for about 5-10% of the total cell protein. The inclusion bodies containing **TFPI** were sulfitolyzed, **purified** by anion-exchange chromatography, refolded through a

disulfide interchange reaction, and further fractionated by Mono S cation exchange chromatography. The Mono S resin resolved a peak of highly active **TFPI** from relatively inactive and possibly misfolded molecules. The E. coli **TFPI** was shown to be about two-fold more active, on a molar basis, than full-length human SK hepatoma **TFPI** in a tissue factor-induced clotting assay in human plasma.

CC Biochemistry methods - Proteins, peptides and amino acids 10054
Biochemistry studies - Proteins, peptides and amino acids 10064
Biophysics - Methods and techniques 10504
Biophysics - Molecular properties and macromolecules 10506
Metabolism - Proteins, peptides and amino acids 13012
Blood - Blood and lymph studies 15002
Endocrine - General 17002
Physiology and biochemistry of bacteria 31000
In vitro cellular and subcellular studies 32600

IT Major Concepts
Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Endocrine System (Chemical Coordination and Homeostasis); Metabolism; Physiology

IT Miscellaneous Descriptors
ANALYTICAL **METHOD**; ANION EXCHANGE CHROMATOGRAPHY

ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia coli
Taxa Notes
Bacteria, Eubacteria, Microorganisms

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

L81 ANSWER 63 OF 67 HCAPLUS COPYRIGHT 2004 ACS on STN
AN 1994:474759 HCAPLUS
DN 121:74759
ED Entered STN: 20 Aug 1994
TI Renaturation and purification of human tissue factor pathway inhibitor expressed recombinant E. coli
AU Gustafson, Mark E.; Junger, Kurt D.; Wun, Tze-Chen; Foy, Barbara A.; Diaz-Collier, Judith A.; Welsch, Dean J.; Obukowicz, Mark G.; Bishop, Bruce F.; Bild, Gary S.; et al.
CS Monsanto Corporate Res., Chesterfield, 63198, USA
SO Protein Expression and Purification (1994), 5(3), 233-41
CODEN: PEXPEJ; ISSN: 1046-5928
DT Journal
LA English
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 13
AB Tissue factor pathway inhibitor is an inhibitor of the extrinsic coagulation pathway. Evaluation of the pharmacol. effects of tissue factor pathway inhibitor in animal models has been limited by the high cost and low availability of mammalian tissue culture produced protein. In order to circumvent this obstacle, a 277-amino-acid nonglycosylated

tissue factor pathway inhibitor variant possessing an N-terminal alanine was expressed in recombinant E. coli using the tac promoter expression system. High-level expression in recombinant E. coli resulted in the accumulation of ala-tissue factor pathway inhibitor in inclusion bodies. Active protein was produced by **solubilization** of the inclusion bodies in 8 M urea, purification of the full-length mol. by cation exchange chromatog., and renaturation in 6 M urea. Fractionation of crude refold mixts. using cation exchange chromatog. yielded a purified nonglycosylated tissue factor pathway inhibitor possessing in vitro prothrombin time activity comparable to inhibitor purified from mammalian cell lines.

ST human tissue factor pathway inhibitor Escherichia; Escherichia expression tissue factor pathway inhibitor

IT Inclusion bodies
(human tissue factor pathway inhibitor expression in Escherichia coli and accumulation in, purification in relation to)

IT Escherichia coli
(human tissue factor pathway inhibitor expression in, accumulation in inclusion bodies after, purification in relation to)

IT **Blood-coagulation factors**
RL: BIOL (Biological study)
(**LACI** (**lipoprotein**-associated **coagulation inhibitor**), Escherichia coli expression of, of human, accumulation in inclusion bodies of, purification in relation to)

IT Genetic element
RL: PRP (Properties)
(promoter, tac, human tissue factor pathway inhibitor expression in Escherichia coli using system of, accumulation in inclusion bodies after, purification in relation to)

IT 56-41-7, Alanine, biological studies
RL: BIOL (Biological study)
(human tissue factor pathway inhibitor variant possessing N-terminal, expression in Escherichia coli of, accumulation in inclusion bodies after, purification in relation to)

IT 9001-26-7, Prothrombin
RL: BIOL (Biological study)
(purified human tissue factor pathway inhibitor expressed in Escherichia coli and possessing activity of, comparable to mammalian cell line inhibitor in relation to)

L81 ANSWER 65 OF 67 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN 1993-175458 [21] WPIX
DNC C1993-078392
TI Production of non-glycosylated form of **tissue factor pathway inhibitor** in high yield - comprises culturing E-coli cells transformed with replication expression vector and subjecting isolated inclusion bodies to sulphitolysis or reduction with beta-mercapto-ethanol, etc..
DC B04 D16
IN DIAZ-COLLIER, J A; GUSTOFSON, M E; WUN, T; GUSTAFSON, M E
PA (MONS) MONSANTO CO
CYC 17

PI	US 5212091	A 19930518 (199321)*	25	C12P021-02	
	EP 559632	A2 19930908 (199336) EN	35	C12N015-15	
	R: AT BE CH DE DK FR GB IE IT LI LU NL PT SE				
	CA 2090650	A 19930903 (199347)		C12N015-12	
	EP 559632	A3 19950329 (199543)		C12P021-02	
	JP 07274968	A 19951024 (199551)	20	C12N015-09	
	EP 559632	B1 19980819 (199837) EN		C12N015-15	
	R: AT BE CH DE DK FR GB IE IT LI LU NL PT SE				
	DE 69320389	E 19980924 (199844)		C12N015-15	

JP 3333846 B2 20021015 (200275) 19 C12N015-09
ADT US 5212091 A US 1992-844297 19920302; EP 559632 A2 EP 1993-870037 19930301; CA 2090650 A CA 1993-2090650 19930301; EP 559632 A3 EP 1993-870037 19930301; JP 07274968 A JP 1993-40210 19930301; EP 559632 B1 EP 1993-870037 19930301; DE 69320389 E DE 1993-620389 19930301, EP 1993-870037 19930301; JP 3333846 B2 JP 1993-40210 19930301
FDT DE 69320389 E Based on EP 559632; JP 3333846 B2 Previous Publ. JP 07274968
PRAI US 1992-844297 19920302
REP No-SR.Pub; 5.Jnl.Ref; EP 219874; EP 361475; EP 361830; WO 9325230
IC ICM C12N015-09; C12N015-12; C12N015-15; C12P021-02
ICS B01D015-08; C07K001-14; C07K003-28; C07K013-00; C07K014-81; C12N001-21
ICA A61K038-00; A61P007-02
ICI C12N001-21, C12R001:19; C12P021-02, C12R001:19; C12N001-21, C12R001:19; C12P021-02, C12R001:19
AB US 5212091 A UPAB: 19931114
Production of a non-glycosylated form of **tissue factor pathway inhibitor** (TFPI) comprises culturing E. coli cells which have been transformed with a replicable expression vector containing the cDNA encoding TFPI, harvesting the E. coli cells, isolating the inclusion bodies from the cells and subjecting the inclusion bodies to a stepwise **purificn.** comprising (A) subjecting the inclusion bodies to sulphitolysis to form TFPI-sulphonate, **purifying** this by anion exchange chromatography, refolding TFPI-S by disulphide interchange reaction, and **purifying** active refolded TFPI by cation exchange chromatography, or (B) subjecting the inclusion bodies to reduction with beta-mercaptoethanol in urea to form reduced TFPI, **purifying** this by cation exchange chromatography, refolding reduced TFPI by disulphide interchange reaction in urea and **purifying** the active refolded TFPI by cation exchange chromatography.

Sulphitolysis is pref. performed by reaction of the inclusion bodies with sodium sulphate and sodium dithionite. Anion exchange is performed by HPLC of the TFPI-S- sulphonate using a quat. amine anion exchange resin. Refolding of TFPI-S-sulphonate is performed by reaction with L-cysteine. Cation exchange chromatography is via HPLC using sulphonic acid gp. cation exchange resin.

USE/ADVANTAGE - TFPI, alternatively known as lipoprotein associated coagulation inhibitor (LACI), may be produced in high yield (over 100 mg/ml) by this method. A greater level of homogeneity of TFPI prod. is obtd..

Dwg.0/12
FS CPI
FA AB
MC CPI: B04-B04A5; B12-G01; D05-C12; D05-H13

L81 ANSWER 67 OF 67 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1993:137088 BIOSIS
DN PREV199395069888
TI Bacterial expression, **purification**, and partial characterization of amino acids 94-155 of human tissue factor pathway inhibitor (**TFPI**) as an inhibitor of blood coagulation factor Xa.
AU Day, Kathleen C.; Welsch, Dean J. [Reprint author]
CS Monsanto Co., 700 Chesterfield Village Parkway, St. Louis, Mo. 63198, USA
SO Thrombosis Research, (1992) Vol. 68, No. 4-5, pp. 369-381.
CODEN: THBRAA. ISSN: 0049-3848.
DT Article
LA English
ED Entered STN: 16 Mar 1993

Last Updated on STN: 17 Mar 1993

AB Tissue factor pathway inhibitor (**TFPI**) is a plasma-derived protein which inhibits two of the active serine proteases present during normal blood coagulation. Inhibition of both of these proteases, factors VIIa and Xa, is thought to require a factor Xa-**TFPI** complex. To begin o investigate the interactions between factor Xa and **TFPI**, amino acids 94-155, which encode for the second Kunitz domain (K2) of **TFPI**, were expressed, **purified**, and partially characterized. Expression of the recombinant peptide was accomplished using an Escherichia coli expression system which produced the peptide at an expression level of approximately 2-5% of total cell protein. The peptide was localized to disulfide-linked refractile bodies which were **solubilized** by reduction in the presence of denaturant and the soluble protein refolded. Oxidized K2 was **purified** from the refold mixture using a two-step procedure employing gel filtration chromatography and reverse-phase HPLC. The unprocessed form of the recombinant peptide, Met-Ala-K2 (rMA-K2), was characterized. This peptide was **purified** to apparent homogeneity as determined by SDS-PAGE, quantitative amino acid, Edman degradation, and electrospray mass spectrometry analyses (gt 95% pure). The product bound to factor Xa covalently coupled to a solid support in the presence of 2M sodium chloride demonstrating its affinity for this enzyme. Preincubation of rMA-K2 peptide with factor Xa neutralized, with 1.1:1 stoichiometry, the ability of factor Xa to hydrolyze a small chromogenic substrate. Additionally, rMA-K2 prolonged the time to clot formation in a plasma-based assay dependent on factor Xa concentration. Finally, this peptide mildly prolonged the prothrombin and modified prothrombin times of normal pooled plasma. Taken together this data demonstrates that this region of **TFPI** inhibits factor Xa activity and allows for further characterization of this enzyme-inhibitor complex.

CC Biochemistry studies - Proteins, peptides and amino acids 10064
Enzymes - Physiological studies 10808
Blood - General and methods 15001
Blood - Blood and lymph studies 15002
Genetics of bacteria and viruses 31500

IT Major Concepts
Blood and Lymphatics (Transport and Circulation); Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
FACTOR XA; PROTHROMBIN; SERINE PROTEASE

IT Miscellaneous Descriptors
CLOT FORMATION; KUNITZ DOMAIN; **METHOD**; PROTHROMBIN; SERINE PROTEASE

ORGN Classifier
Enterobacteriaceae 06702
Super Taxa
Facultatively Anaerobic Gram-Negative Rods; Eubacteria; Bacteria; Microorganisms
Organism Name
Escherichia coli
Taxa Notes
Bacteria, Eubacteria, Microorganisms

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
Hominidae
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 9002-05-5 (FACTOR XA)
9001-26-7 (PROTHROMBIN)
37259-58-8 (SERINE PROTEASE)

=> b home
FILE 'HOME' ENTERED AT 17:32:02 ON 08 JUL 2004

=> log h

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	151.00

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	0.00	-1.47

SESSION WILL BE HELD FOR 60 MINUTES
STN INTERNATIONAL SESSION SUSPENDED AT 17:32:05 ON 08 JUL 2004